

Appendix 7-9. Annual Permit Compliance Monitoring Report for Mercury in Stormwater Treatment Areas and Downstream Receiving Waters of the Everglades Protection Area

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INTRODUCTION

This is the third Annual Permit Compliance Monitoring Report for Mercury in Stormwater Treatment Areas (STAs) and the Downstream Receiving Waters. This report summarizes the mercury-related reporting requirements of the U.S. Army Corps of Engineers (USACOE) Section 404 Dredge and Fill Permit (Permit No.199404532), the Florida Department of Environmental Protection (FDEP) NPDES Permit (FL0177962-001) and FDEP Everglades Forever Act Permits (EFA- Ch. 373.4592, F.S). The latter includes permits for Non-Everglades Construction Project Discharge Structures, STA-6, STA-5, STA-1 West, and STA-2 (No. 06,502590709, 262918309, 0131842, FL0177962-001, 0126704). This Report summarizes the results of monitoring in the reporting year ending April 30, 2000. Because STA-6 has been in operation for two full years, in accordance with Condition 8.b.(4) of the USACOE 404 permit, this assessment of mercury storage, release and bioaccumulation will be based on the first two full years of data.

The Report consists of an Introduction, Background, Summary of the Mercury Monitoring and Reporting Program, Monitoring Results and, Key Findings and Overall Assessment. The Background section briefly summarizes the operation of the STAs and discusses their possible impact on south Florida's mercury problem. This section also includes site descriptions and maps of each STA that is currently monitored (in the order that they became operational). The next section summarizes both sampling and reporting requirements of the mercury-monitoring program. Monitoring results are summarized and discussed in three subsections: (1) results from pre-operational monitoring, (2) results from STA operational monitoring, and (3) results from monitoring downstream receiving

waters. Recent results from the Mercury Monitoring Program describe significant spatial distributions and, in some instances, between-year differences in mercury concentrations. The final section summarizes key findings and presents an overall assessment of mercury impacts within and downstream of the STAs, with a focus on STA-6.

BACKGROUND

The STAs are treatment marshes designed to remove nutrients from stormwater runoff originating from upstream agricultural areas. The STAs are being built as part of the Everglades Construction Project (ECP). When completed, the ECP will include six STAs totaling about 43,000 acres of constructed wetlands. The downstream receiving waters to be restored and protected by the ECP include the District's water management canals of the Central and Southern Florida (C&SF) Project and the interior marshes of the Everglade Protection Area, encompassing Water Conservation Areas (WCA) 1, 2, and 3, and the Everglades National Park (ENP).

Concerns were raised that in reducing downstream eutrophication, this restoration effort might inadvertently worsen the Everglades mercury problem (FGMFWTF, 1991). Widespread elevated concentrations of mercury were first discovered in freshwater fish from the Florida Everglades in 1989 (Ware et al., 1990). Mercury is a persistent, bioaccumulative toxic pollutant. Consequently, mercury can build up in the food chain to levels that are harmful to human and ecosystem health. Based on the levels observed in 1989, state fish consumption advisories were issued for select species and locations (Florida Department of Health and Rehabilitative Services and Florida Game and Fresh Water Fish Commission, March 6, 1989). Subsequently, elevated concentrations of mercury have also been found in predators like raccoons (Florida Panther Interagency Committee, 1991), alligators (Heaton-Jones et al., 1997; Jagoe et al., 1998), Florida panthers (Roelke and Glass, 1992) and wading birds (Sundlof et al., 1994).

To provide assurance that the ECP is not exacerbating the mercury problem, the South Florida Water Management District (SFWMD) monitors concentrations of total mercury (THg) and methylmercury (MeHg) in various abiotic (e.g., water and sediment) and biotic (e.g., fish and bird tissues) media. There are a number of advantages of monitoring mercury concentrations in biota. First, MeHg occurs at much greater concentration as residues in biota relative to concentrations in water, which makes chemical analysis more accurate and precise. Although detection levels of part per trillion (ppt or ng/L) or even part per quadrillion have been achieved for THg and MeHg in water, uncertainty boundaries can become increasingly large when ambient concentrations are very low, as is often the case in the Everglades. Second, organisms integrate exposure to mercury over space and time. This is key because concentrations in surface water can vary dramatically over small spatial and temporal scales. For example, THg and MeHg in the water column both show substantial diel trends in concentrations (Krabbenhoft et al., 1998). Finally, tissue mercury concentration is a true measure of MeHg bioavailability and is a better indicator of possible negative effects than total amount of mercury in the environment.

SITE DESCRIPTIONS

STA-6

STA-6, Section 1 is located at the southeastern corner of Hendry County and southwest corner of the EAA. STA-6, Section 1 has two treatment cells (Cell 5=252 ha and Cell 3=99 ha) designed to provide a total effective treatment area of 352 ha (870 acres, **Figure A7-9-1**, for additional details, see SFWMD, 1997a). The United States Sugar Corporation, (USSC), has operated the two cells as a storm water retention area since 1989. Approximately 4,210 ha of USSC's agricultural production area (Southern Division Ranch, Unit 2) drains into STA-6, Section 1 via a Supply Canal and existing pump station, G600, that continues to be under the operation of USSC. Water flows from the Supply Canal to the treatment cells via inflow weirs (two for Cell 5 and one for Cell 3). Water then flows in an easterly direction and is discharged through six recently installed culverts (G-354 A-C for Cell 5 and G-393 A-C for Cell 3) each with a fixed crest weir at 13.6 ft NGVD to limit drawdown of each treatment cell to the desired static water level of 13.6 ft NGVD (maximum combined discharge of 500 cfs). This outfall then enters the Discharge Canal, which gravity discharges to the L-4 borrow canal via six culverts, which are confluent to G607. The L-4 Borrow Canal conveys flows eastward to the S-8 pump station, which discharges into Water Conservation Area 3A. Upon demand, water can be conveyed from L-4 canal backward (using stop logs at G604 to bypass flows to the L-4 from the G607 culverts) to USSC Unit 2 farm for irrigation. As a consequence, unlike other STAs, timing, quantity, duration of inflows and backflows, and thus mean depth, hydraulic loading rate and hydraulic residence time (HDT) of STA-6 are controlled by USSC via the operation of G600.

STA-5

STA-5 is immediately north of USSC's Southern Division Ranch, Unit 2, and extends from the L-3 levee on the west to the Rotenberger Tract on the east. STA-5 consists of two parallel treatment cells, Cell 1 and Cell 2, to provide a total effective treatment area of 1666 ha (4,118 acres, **Figure A7-9-2**, for additional details, see SFWMD, 1998a). Under typical operations, water from the L-3 Borrow canal, the Deer Fence Canal and the S&M Canal will gravity flow into the two treatment cells through four gated inflow culverts (G342A, G342B, G342C, G342D). Water will continue to gravity flow east through the western portions of the treatment area through eight open culverts into the eastern treatment areas; each treatment cell is subdivided by an internal levee because of a significant downward slope in ground elevation from west to east. Water will then gravity flow through four discharge structures (G344A, B for Treatment Cell 1 and G344C, D for treatment Cell 2) and then discharge into STA-5 discharge canal. The STA-5 discharge canal continues along the western and northern sides of the Rotenberger Wildlife Management Area, ultimately emptying into the Miami Canal. However, direct discharge to the Rotenberger tract are possible and maybe used to supplement the natural accumulation of water via rainwater on an as-needed basis.

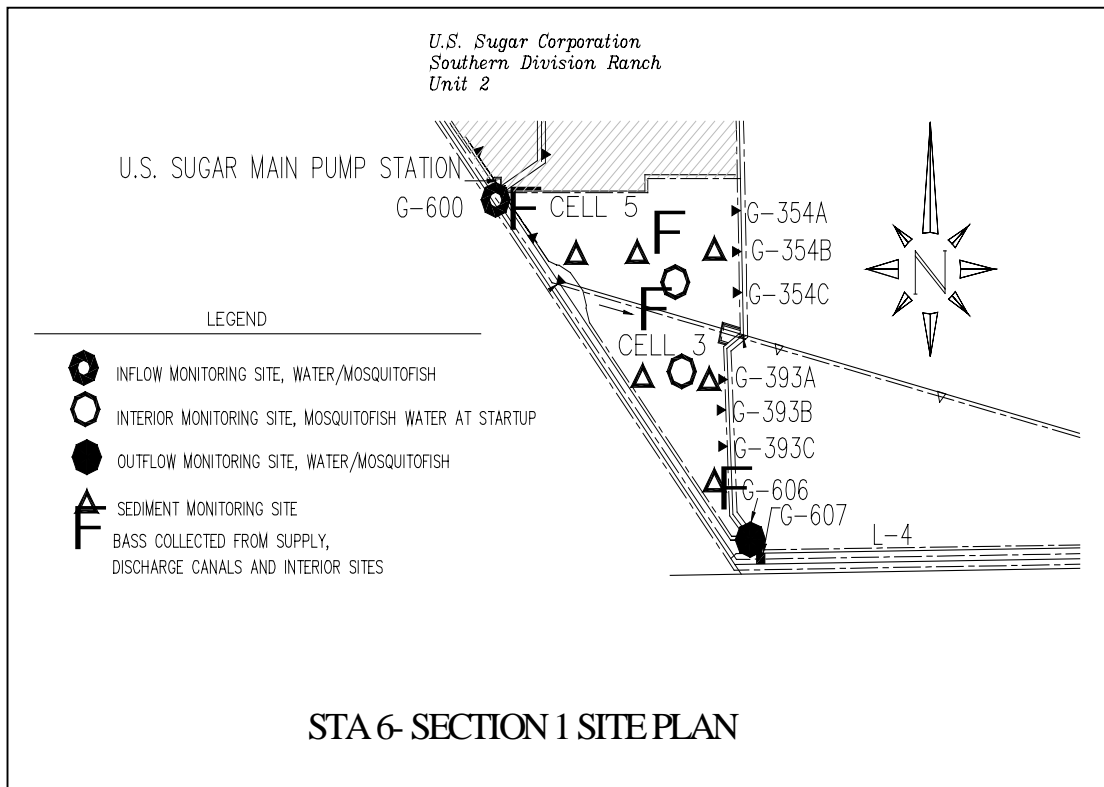


Figure A7-9-1. Map of STA-6.

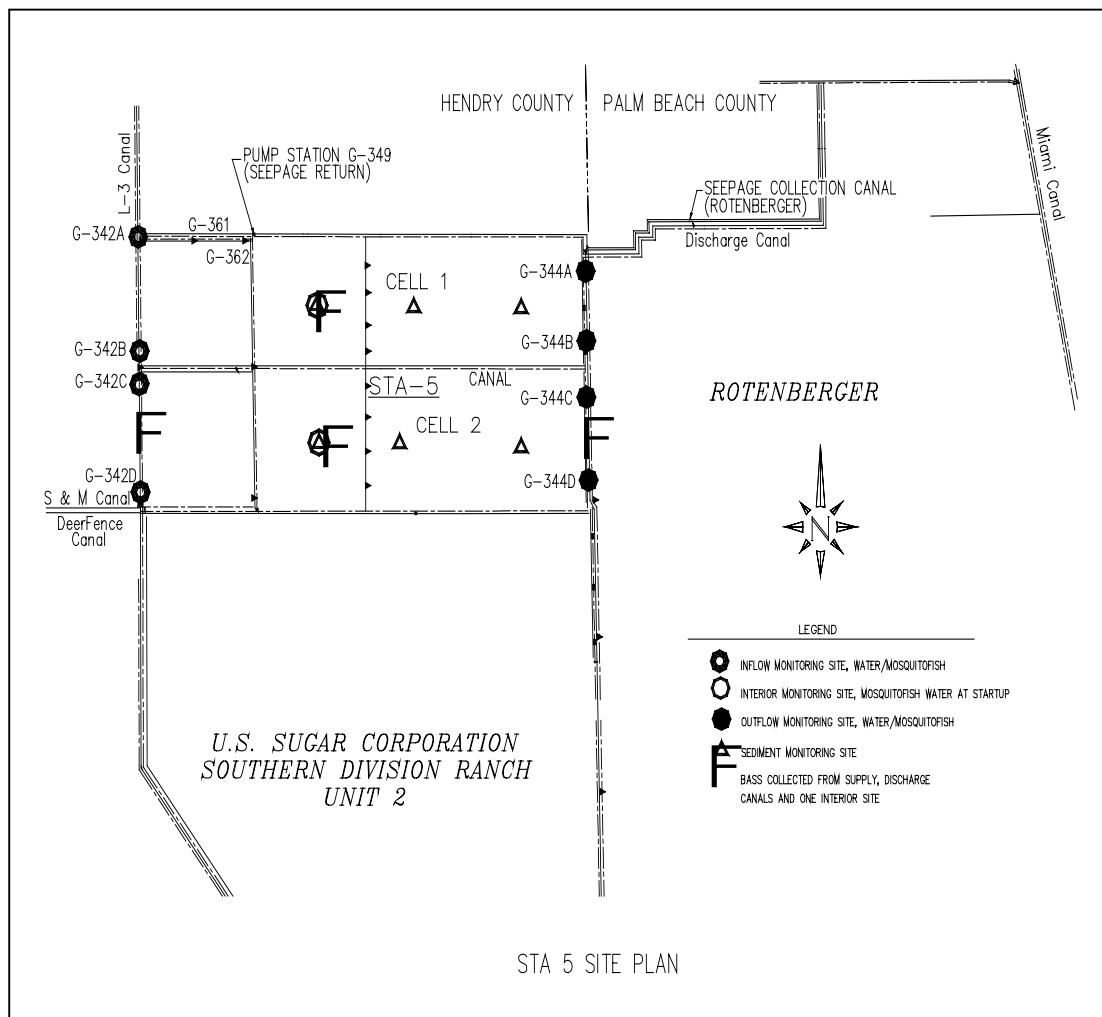


Figure A7-9-2. Map of STA-5.

STA-1 West

STA-1 West is located in western Palm Beach County, immediately west of the Arthur R. Marshall Loxahatchee National Wildlife Refuge (WCA-1). STA-1W is designed to provide a total effective treatment area of 6,870 acres, including the 3,815 acres of the existing Everglades Nutrient Removal (ENR) Project, which it subsumes in April 1999 (**Figure A7-9-3**, for additional details, see SFWMD, 1998b). Under typical operations, S5A basin runoff is conveyed to STA-1W from pump station S5A via STA-Inflow and Distribution Works gated weir structure G302. Flows will travel in a southwesterly direction via the inflow canal into Treatment Cell 5 via culverts G304 A-J and into Treatment Cells 1 through 4 (existing ENR Project) via gated weir structure G303. Flows through Cell 5 are conveyed in a westerly direction through structures G305 A-V and discharged through culverts G306 A-J into the discharge canal. This discharge is then conveyed to WCA-1 via this canal and pump station G310. Flows through Treatment Cells 1 through 4 are conveyed in a southerly direction through G252 and G253 (Cells 1 and 3) and G254, G255 and G256 (Cells 2 and 4). Flows are discharged into WCA-1 via existing ENR Project collection canals and existing pump station G251, and under some conditions (when ENR Project outflows exceed G251 pump capacity of 450 cfs), through structures G258, G259, G308 and G309 into discharge canal and pump station G310. Thus, there are two primary discharge locations for STA-1W into the L-7 Canal located in the Refuge.

STA-2

STA-2 is located in western Palm Beach County near the Browns Farm Wildlife Management Area. STA-2 will be developed to provide a total effective treatment area of 6,430 acres (for additional details, see SFWMD, 1999a). It is intended to treat discharges from the S-6/S-2 Basin, S-5A Basin, East Shore Water Control District, 715 Farms and Lake Okeechobee via pump stations S-6 and G328. S-6 will serve as the primary inflow pumping station, with G328 serving as both an irrigation and “secondary” inflow source from and to the STA supply canal (**Figure A7-9-4**). Discharges from the supply canal are then conveyed southward to the inflow canal, which extends across the northern perimeter. A series of inflow culverts will convey flows from the inflow canal to the respective treatment cells (G329 A-D into Cell 1, G331 A-G into Cell 2, G333 A-E into Cell 3). Flows will travel southward through the treatment cell eventually discharging to the discharge canal via culverts or gated spillways (culverts G330 A-E from Cell 1, gated spillway G332 from Cell 2, gated spillway G334 from Cell 3). Flows will then travel eastward in the discharge canal to the STA-2 outflow pump station G335, which in turn conveys water to the L-6 Borrow canal. Water from the L-6 Borrow canal will sheet flow across the L-6 levee into northwestern WCA-2A via uncontrolled spillways G336 A-NN.

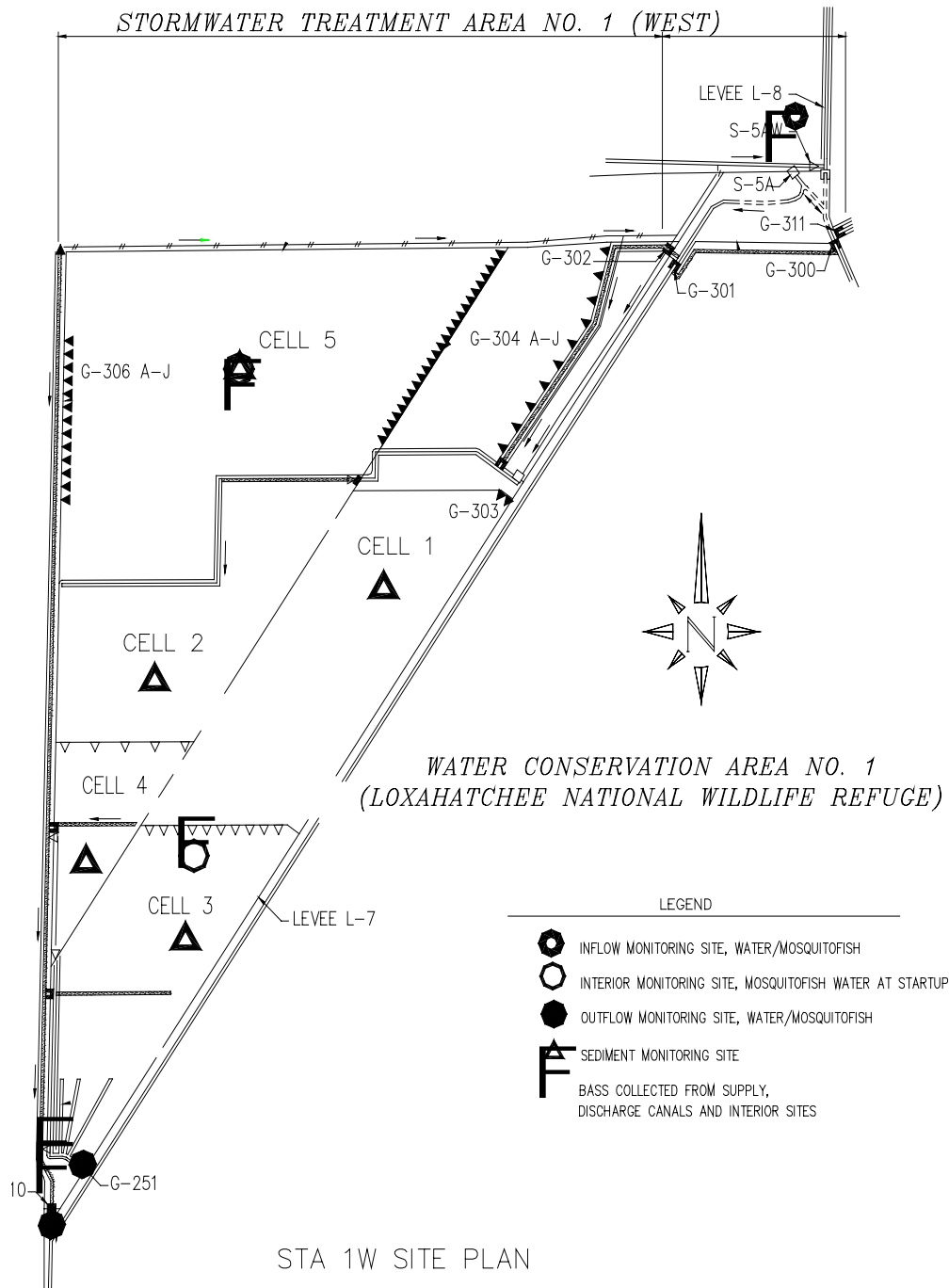


Figure A7-9-3. Map of STA-1W.

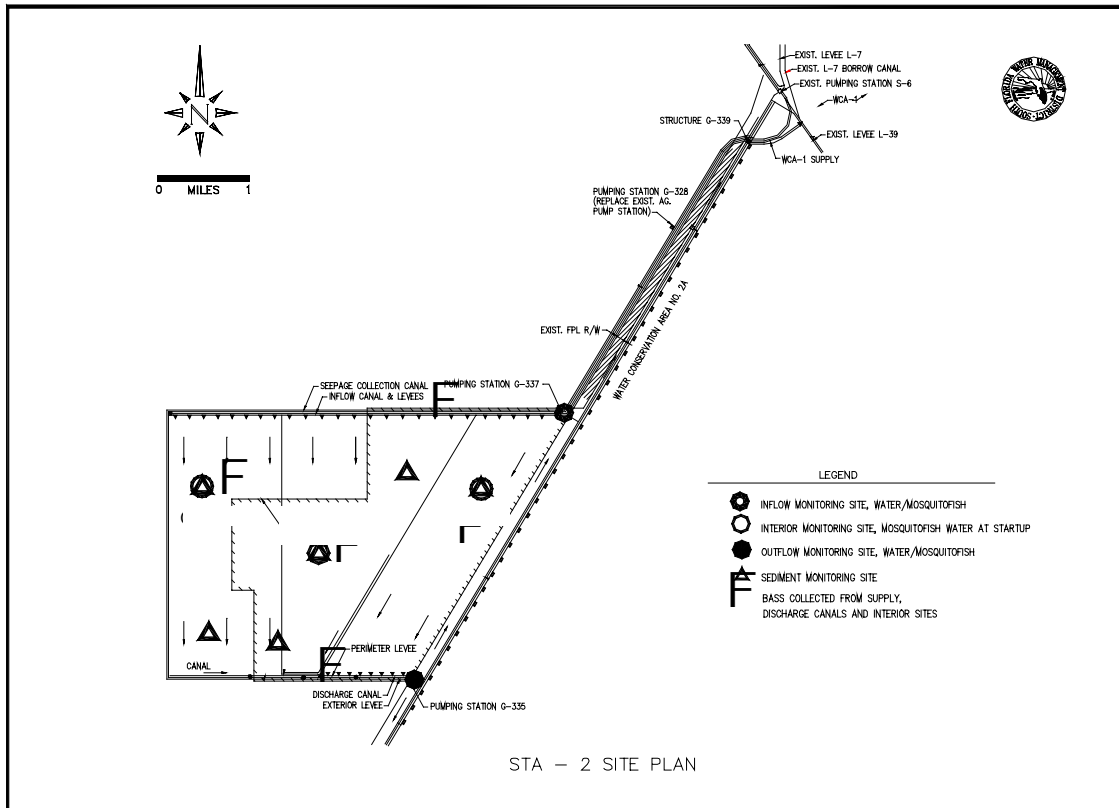


Figure A7-9-4. Map of STA-2

SUMMARY OF THE MERCURY MONITORING AND REPORTING PROGRAM

The monitoring and reporting program summarized below is described in detail in the “Mercury Monitoring and Reporting Plan for the Everglades Construction Project, the Central and Southern Florida Project, and the Everglades Protection Area”, which was submitted by the District to the Florida Department of Environmental Protection, the U.S. Environmental Protection Agency, and the U.S. Army Corps of Engineers, in compliance with the requirements of the aforementioned permits. The details of the procedures to be used in ensuring the quality of and accountability for the data generated in this monitoring program are set forth in the District’s “Quality Assurance Project Plan (QAPP) for the Mercury Monitoring and Reporting Program”, which was approved upon issuance of the permit by the Florida Department of Environmental Protection (FDEP). QAPP revisions were approved by FDEP on June 7, 1999.

EVERGLADES MERCURY BASELINE MONITORING AND REPORTING REQUIREMENTS

Levels of THg and MeHg in the pre-operational soils of each of the STAs and various compartments (i.e., media) of the downstream receiving waters define the baseline condition from which to evaluate the mercury-related changes, if any, brought about by the operation of the STAs. The pre-ECP mercury baseline conditions are defined in the Everglades Mercury Background Report, which summarized all of the relevant mercury studies conducted in the Everglades through July 1997, during the construction but prior to the operation of the first STA. Originally prepared for submittal in February 1998, it has now been revised to include the most recent data released by the U.S. Environmental Protection Agency and U.S. Geological Survey and was submitted in February 1999 (FTN Associates, 1999)

PRE-OPERATIONAL MONITORING AND REPORTING REQUIREMENTS

Prior to completion of construction and flooding of the soils of each STA, the District is required to collect and analyze 10-cm core samples of soil at six representative interior sites and analyze them for THg and MeHg. Prior to initiation of discharge, the District is also required to collect biweekly samples of inflow and interior water for analysis for THg and MeHg concentrations. When concentrations at the interior sites are observed to be less than that of the inflow, this information is reported to the permit-issuing authority and the biweekly sampling can be discontinued. Discharge begins after all of the start-up criteria are met.

This is followed by a two-year stabilization period for both phosphorus and mercury. During this stabilization period, the release of stored phosphorus and mercury from flooded farm fields soils is anticipated, with concomitant instances of outflow or interior concentrations exceeding inflow concentrations. As the bioavailable phosphorus and mercury are transformed from the soil reservoir to the colonizing plants and accreting

marsh soils, the magnitude, duration, and frequency of such phenomena will decrease until stabilization is achieved and the outflow and interior concentrations are routinely less than the inflow.

OPERATIONAL MONITORING

STAs

Following approval for initiation of routine operation of the facility and thereafter, the permits require that the following samples be collected at the specified frequencies and analyzed for the specified analytes:

Water: Quarterly, 500-ml unfiltered grab samples of water will be collected in pre-cleaned Teflon bottles using ultra-clean technique at the inflows and outflows of each STA and analyzed for THg and MeHg. THg results will be compared with the Florida Class III Water Quality Standard of 12 ng/L to ensure compliance. Outflow concentrations of both THg and MeHg will be compared to concentrations at the inflow.

Sediment: Triennially, six, 10-cm sediment cores will be collected at representative interior sites and homogenized. The homogenate will be analyzed for THg and MeHg.

Preyfish: Semi-annually a grab sample of between 100 and 250 preyfish will be collected using a dipnet at the inflow sites, an interior site, and the outflow sites of each STA and homogenized. The homogenate is to be subsampled in quintuplicate and each subsample analyzed for THg. Typically, the preyfish will be primarily composed of mosquitofish (*Gambusia sp.*). This species has been selected as a representative indicator of short-term, localized changes in water quality because of its small range, short life span and wide occurrence in the Everglades.

Top-predator Fish: Annually 20 largemouth bass of a legally harvestable size will be collected primarily via electroshocking methods at representative inflow and outflow sites and a representative interior site in each STA and the fish muscle will be analyzed for THg as an indicator of potential human exposure.

It is important to note that 85 to 99% of the THg in mosquitofish (*Gambusia sp.*) is MeHg (Grieb et al., 1990; R. Jones, FIU, pers. comm., 1995; L. Cleckner, University of Wisconsin, pers. comm, 1996; SFWMD, unpublished data) and more than 95% of the THg in higher trophic level fish is MeHg (Watras, 1993). Therefore, the analysis of fish tissue for THg is interpreted as equivalent to the analysis of fish tissue for MeHg for the purposes of this report.

Downstream Receiving Water

The downstream system is monitored to track changes in mercury concentrations over space and time in response to the changes in hydrology and water quality brought about by the ECP (for site locations, refer to **Figures A7-9-5 and -6**).

Rain Water: From 1992 to 1996, the District, FDEP, USEPA, and a consortium of southeastern U.S. power companies sponsored the Florida Atmospheric

Mercury Study (FAMS). FAMS results in comparison with monitoring of surface water inputs to the Everglades showed that >95% of the annual mercury budget came from rain. It was clear that the major source of mercury to the Everglades was from the air. Accordingly, the District continues to monitor atmospheric wet-deposition to monitor inputs. Weekly, the volume of bulk rainfall collected with a polycarbonate funnel and accumulated weekly in a two-liter Teflon bottle at the top of 48-ft. towers at the Everglades Nutrient Removal (ENR) Project, Andytown substation of Florida Power and Light (I-75/U.S.27) and Everglades National Park will be analyzed for THg as part of the National Atmospheric Deposition Program's Mercury Deposition Network.

District Structures Surface Water: Quarterly, 500-ml unfiltered grab samples of water will be collected in pre-cleaned Teflon bottles using ultra-clean technique upstream of the following structures and analyzed for THg and MeHg: S-5A, S-10C, S-140, S-9, S-32, S-151, S-141, S-190/L-28 interceptor, S-334, and S-12D. These sites bracket the WCAs or are major points of inflow or outflow. Monitoring these sites should therefore capture the effect of seasonal changes in the relative contributions of rainfall and stormwater runoff contributing to water quality entering the EPA.

Preyfish: Semi-annually, a grab sample of between 100 and 250 preyfish will be collected using a dipnet at 12 downstream interior marsh sites, homogenized, and the homogenate subsampled in quintuplicate and each subsample analyzed for THg. Typically, the preyfish will be primarily composed of mosquitofish (*Gambusia sp.*). This species has been selected as a representative indicator of short-term, localized changes in water quality because of its small range, short life span and wide occurrence in the Everglades.

Secondary Predator Fish: Annually, 20 fish in the genus *Lepomis* (sunfish species) will be collected at twelve downstream interior marsh sites, and each whole fish will be analyzed for THg. Because of its wide occurrence and because it is a preferred prey species, *Lepomis* was selected as an indicator of the exposure to wading birds.

Top-Predator Fish: Annually, 20 largemouth bass (*Micropterus salmoides*) of a harvestable size will be collected primarily via electroshocking methods at 12 downstream interior marsh sites and the muscle will be analyzed for THg. Largemouth bass were selected as an indicator of potential human exposure.

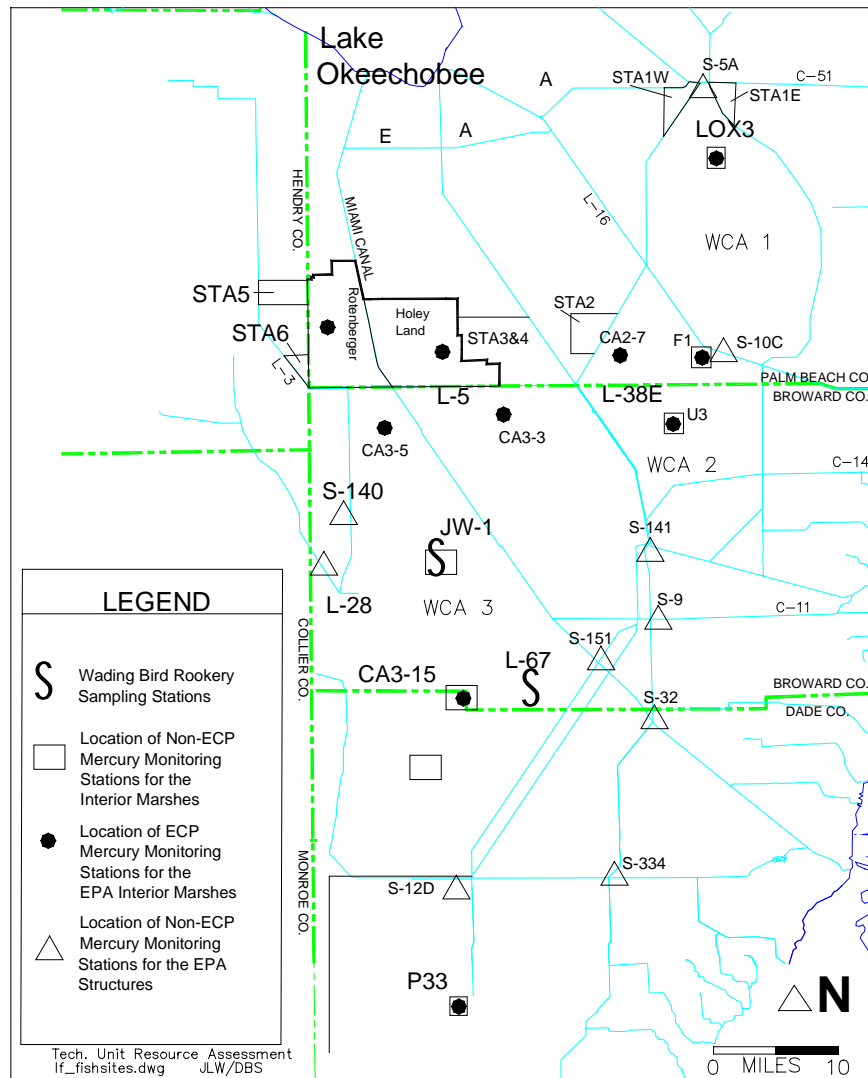


Figure A7-9-5. Downstream canal and interior marsh monitoring stations for water, fish and bird feathers.

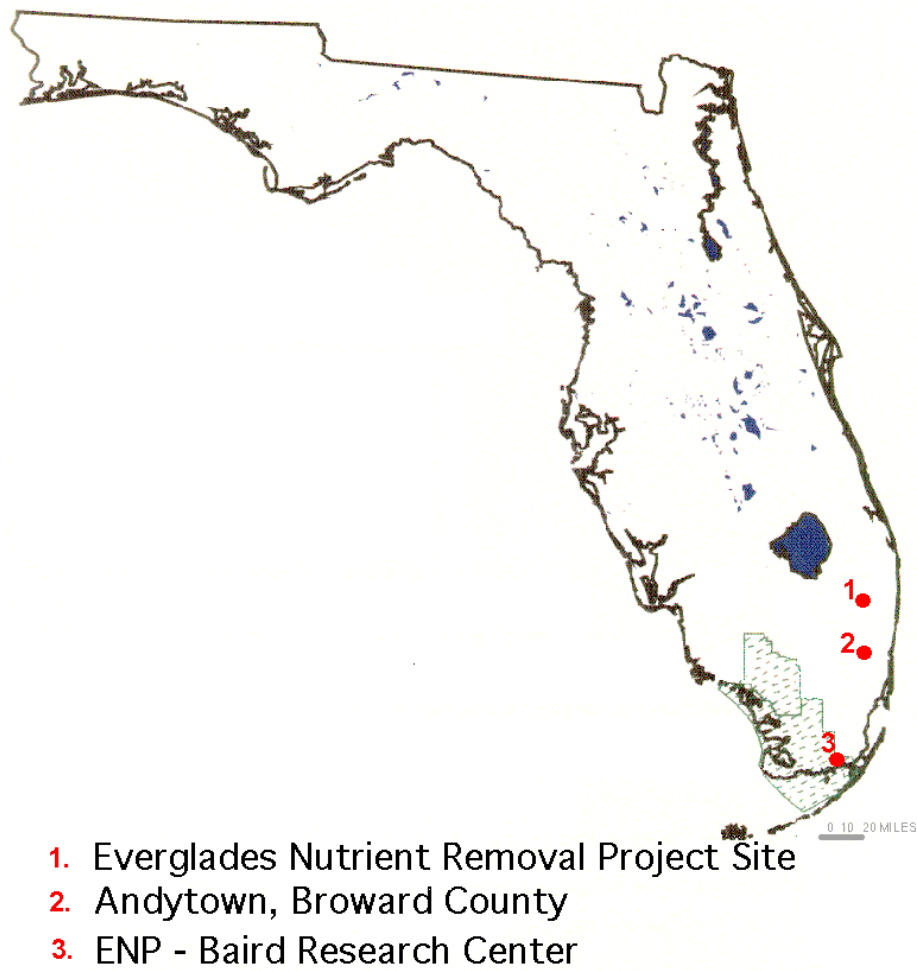


Figure A7-9-6. Mercury deposition network in South Florida.

Feathers: Annually, feathers will be collected from 20 great egret nestlings from two different nesting colonies within WCA-3A and analyzed for THg under appropriate state and federal permits (WX99076, MB007948-0). Because MeHg bioaccumulates in top-predator fish, the organisms most highly exposed in the Everglades are the fish-eating birds, including the wading birds. [Note, this is a modification from the initial sampling design, which would have involved collecting molted feathers from post-breeding adults as they lay at or in the immediate vicinity of nests or from STAs. This modified sampling design is more consistent with protocols used in the collection of background data (Frederick et al., 1997).]

In addition to the monitoring program described above, in accordance with Condition 4.iv of the Mercury Monitoring Program, the District is required to “report changes in wading bird habitat and foraging patterns using data collected in on-going studies conducted by the permittee and other agencies.”

Further details regarding rationales for sampling scheme, procedures and data reporting requirements are set forth in the Everglades Mercury Monitoring Plan revised March 1999 (Appendix 1 of QAPP, June 7, 1999).

Statistical Methods

Temporal trends in water column THg and MeHg concentrations were evaluated using the Mann-Kendall test (for a review see Gilbert, 1987). This procedure is useful because data need not conform to any particular distribution and data reported as less than detection limit can be used. In the future, when additional valid data become available, a Seasonal Kendall Test (Gilbert, 1987) will be used to evaluate this data set.

To be consistent with background data (Lange et al., 1998; 1999; Frederick et al., 1997; Sepulveda et al., 1999), concentrations of mercury in biota were standardized based on animal age or an age surrogate. Standardization based on these metrics is a common practice (Wren and MacCrimmon, 1986; Hakanson, 1980). To be consistent with the reporting protocol used by FFWCC, mercury concentrations in largemouth bass were standardized to an expected mean concentration in 3-year-old fish at a given site by regressing mercury against age (for details see Lange et al., 1999 and references therein). Note, to adjust for month of collection, otolith ages were first converted to decimal age using protocols developed by Lange et al. (1999). This was not done for sunfish, because they were not aged. Instead, arithmetic means were reported. To be consistent with the reporting protocol of Frederick et al. (1997; also see Sepulveda et al., 1999), THg concentrations in nestling feathers were similarly standardized for each site and expressed as least square means for a chick with 7.1 cm bill. Where appropriate, analysis of covariance (ANCOVA; SAS GLM procedure) was used to evaluate spatial and temporal differences in mercury concentrations, with age (largemouth bass), weight (sunfish) or bill size (egret nestlings) as a covariate. However, use of ANCOVA is predicated on several critical assumptions (for review see ZAR, 1996), including (1) that regressions are simple linear functions, (2) that regressions are statistically significant (i.e., non-zero slopes), (3) that the covariate is a random fixed variable, (4) that both the dependent variable and residuals are independent and normally distributed, and (5) that slopes of regressions are homogeneous (parallel). Where these assumptions were not met,

standard ANOVAs or Student's t-tests (SigmaStat, Jandel Corporation, San Rafael, California) were used; possible covariates were considered separately. The assumptions of normality and equal variance were tested by the Kolmogorov-Smirnov and Levene Median tests, respectively. Data sets lacking homogeneity of variance or that departed from normal distribution were natural-log transformed and re-analyzed. If transformed data met the assumptions, they were used in ANOVA. If not, raw data sets were evaluated using non-parametric Mann-Whitney Rank sum tests. If the multi-group null hypothesis was rejected, groups were compared using either Tukey HSD or Dunn's method.

MONITORING RESULTS

PRE-OPERATIONAL MONITORING

STA-6, Section 1

Results from Pre-Operational core samples taken at STA-6, Section 1 were reported in a previous annual report (Rumbold and Rawlik, 2000) and are included here for comparative purposes only (**Table A7-9-1**).

STA-6, Section 1 met start-up criteria for mercury (i.e., concentration of THg and MeHg in the water column of the interior was less than inflow) in November 1997 and began operation in December 1997.

STA-5

Results from Pre-Operational core samples taken at STA-5 were reported in the previous annual report (Rumbold and Rawlik, 2000). Mean THg concentration was 89.4 ± 23.6 ng/g dry weight; mean MeHg was 0.53 ± 0.22 ng/g dry weight. These values were comparable to corresponding concentrations in soils collected in January, 1995 from the ENR Project (127 ng/g THg, 0.2 ng/g MeHg; SFWMD, 1997b).

STA-5 met start-up criteria for mercury (i.e., concentration of THg and MeHg in the water column at the interior was less than inflow) in September 1999.

STA-1 West

Results from pre-operational core samples taken at STA-1W were reported in the previous annual report (Rumbold and Rawlik, 2000). Mean THg concentration was 106.6 ± 27.3 ng/g dry weight; MeHg concentrations in sediments from STA-1W were mostly below the limit of detection; however, this may be a result of poor recovery in analytical laboratory using a new method. If MeHg becomes an issue at this STA, archived samples are available for reanalysis.

STA-1W met start-up criteria for mercury (i.e., concentration of THg and MeHg in the water column at the interior was less than inflow) in January 2000.

Table A7-9-1. Total mercury (THg) and methylmercury (MeHg) concentration in STA soils (i.e., 10-cm depth composited; unit ng/g dry weight).

STA	Year	Sample No.	THg	remark*	MeHg	remark*	% MeHg
STA 6	Sep-97	Cell 5 - 1	87		0.341		0.4
	Sep-97	Cell 5 - 2	31		0.121		0.4
	Sep-97	Cell 5 - 3	24 A		0.192		0.8
	Sep-97	Cell 3 - 4	44		1.093		2.5
	Sep-97	Cell 3 - 5	130		1.373		1.1
	Sep-97	Cell 3 - 6	140		1.496 A		1.1
		mean	76 ±51		0.77 ±0.6		1.2
STA 6	Jan-00	Cell3-SS1	66		0.128		0.2
	Jan-00	Cell3-SS2	78		0.535		0.7
	Jan-00	Cell3-SS3	120		1.751		1.5
	Jan-00	Cell5-SS4	52		0.226		0.4
	Jan-00	Cell5-SS5	21 I		0.178		0.8
	Jan-00	Cell5-SS6	25 I		0.333		1.3
		mean	60 ±37		0.55 ±0.6		1.6
STA 2	April-99	00002	41 A		0.429 A		1.0
	April-99	00003	87		3.172		3.6
	April-99	00004	72		0.236		0.3
	April-99	00005	103		1.948		1.9
	April-99	00006	102		2.614		2.6
	April-99	00008	139		5.008		3.6
		mean	91 ±33		2.235 ±1.79		2.4

* For qualifier definitions, see FDEP rule 62-160. Qualifiers: "A" - averaged value; "U" - undetected, value is the MDL; "I" - below PQL; "J" - estimated value, the reported value failed to meet established QC criteria; "J3" -estimated value, poor precision; "J4" - estimated value, poor recovery in matrix spike or SRM; "?" - do not use, unacceptable field QC, e.g., blank contamination.

STA-2

Six, 10-cm sediment cores were collected from STA-2 on April 21 1999, homogenized, and analyzed for THg and MeHg (**Table A7-9-1**). The mean and standard deviation of sediment concentrations of THg (91 ± 33 ng/g dry weight) are within the expected range of formerly farmed and “virgin” Everglades soils (Delfino et al., 1993) and were similar to THg concentrations in soils collected in January, 1995 from the ENR Project (SFWMD, 1997b). Concentrations of MeHg (2.235 ± 1.79 ng/g dry weight) were highly variable and varied outside the range of what was observed in cores taken from the ENR Project in 1995 (SFWMD, 1997b). The maximum MeHg concentration was also at the extreme range of previously reported concentrations in sediments from the WCAs (Gilmour et al., 1998). MeHg as a percent of THg (%MeHg) was highly variable. Recently, Gilmour et al. (1998) reported %MeHg in sediments of up to 3%. Percent MeHg is considered to be a measure of *in situ* production; where %MeHg is relatively high, the increase in absolute MeHg concentration is thought to be driven by factors other than THg concentration.

As of April 30, 2000, biweekly surface water sample collection has not been started at the inflow sites or interior marsh of STA-2. This will occur following flooding and initial vegetation growth period but prior to discharge. Initiation of discharge will be contingent upon STA-2 meeting the start-up criterion that the concentration of unfiltered THg and MeHg in interior marsh water are not significantly greater than the concentration in corresponding samples of inflow water.

OPERATIONAL MONITORING

STAs

STA-6

As mentioned previously, STA-6 began discharging in December 1997 and, thus, has been in operation for a full two years. Therefore, in accordance with Condition 8.b.(4) of the USACOE 404 permit, this assessment of the effects of construction and operation of this STA and downstream water quality improvements on mercury species storage, release and bioaccumulation will be based on the first two full years of data.

To facilitate this two-year evaluation, sediment cores, which by state permit and USACOE approved Everglades Mercury Monitoring Plan, were to be collected triennially, were collected from STA-6 in January 2000 after only two years (**Table A7-9-1**). Observed concentrations in sediments from both 1997 and 2000 were within the expected range for formerly farmed Everglades soils (Delfino et al., 1993, Gilmour et al., 1998). For example, mean THg in soils from the ENR Project, Water Conservation Areas (WCAs) 1, 2, and 3 and Everglades National Park ranged from 58 to 243 ng/g (Delfino et al., 1993). MeHg concentrations in sediments have been reported to range from less than 0.1 ng/g in the ENR Project to 5 ng/g in WCA-3A (Gilmour et al., 1998).

When the results for recently collected cores were compared to baseline data from cores collected prior to flooding (**Table A7-9-1**), both THg and MeHg were found to be at lower concentration in 2000. This suggests that a small fraction of the mercury mass stored in the peat soil was released when flooded. However, lacking detailed information

on possible changes in bulk densities over two-year period, this conclusion is tentative. In any event, the observed difference in concentrations was not statistically significant for either THg (ANOVA, $df=1,11$; $F=0.375$; $p=0.55$) or MeHg ($df=1,11$; $F=0.385$, $p=0.0.55$).

Interestingly, when the two cells were evaluated separately, sediment concentrations were greater in STA-6 Cell 3 as compared to Cell 5 in both THg (two-way ANOVA evaluating year and cell effects; $df=1, 8$; $F=7.52$; $p=0.025$) and MeHg ($df=1, 8$; $F=9.83$; $p=0.014$). There was no statistically significant interaction between year and cell ($df=1,8$; $F=1.34$; $p=0.28$). In other words, the between-cell difference was established prior to flooding of the STA. As discussed below (and in **Appendix 7-13**), Cell 3 also differs from Cell 5 in terms of tissue mercury concentrations in fish.

Results from operational monitoring of mercury concentrations in STA-6 surface waters are summarized in **Tables A7-9-2** and **A7-9-3**, and graphically presented in **Figure A7-9-7**. As evident from the tables, the Florida Class III Water Quality Standard of 12 ng THg/L was never exceeded at either the inflow or the outflow over the two-year period (i.e., nine quarterly samples). Furthermore, concentrations of both THg and MeHg were within the typical range measured previously at the Everglades Nutrient Removal Project (SFWMD, 1999b). Nevertheless, as discussed in previous reports, on at least two occasions during the first year of operation, THg in surface water was at greater concentration at the outflow compared to the inflow. However, such occurrences are to be expected during the stabilization period. During the current reporting year, both THg and MeHg were lower in concentration in outflow compared to inflow. The percent of THg as MeHg was highly variable in water at both the inflow and outflow ranging from 6.8 to 28.6% (**Table A7-9-2**). This range in %MeHg was consistent with previously reported values for %MeHg in WCA2A and WCA2B (Hurley et al., 1998). Percent change of THg concentration across the STA appears to be stabilized at about 41% (ranged from 30 to 55%, **Table A7-9-3**), with THg in outflow water (tracking) at lower concentration than inflow. Percent change of MeHg concentration across STA-6 was more variable during the reporting year ranging from 0% to 77% (average percent change during the last 4 quarters was 28%, **Table A7-9-3**).

Results from operational monitoring of mercury concentrations in STA-6 fish are summarized in **Tables A7-9-4** and **A7-9-5**, and graphically presented in **Figure A7-9-8**. Levels of mercury in STA-6 mosquitofish have remained comparable to concentrations observed in mosquitofish from the ENR Project (SFWMD, 1999b). In particular, following the decline in the second half of 1998, mercury concentrations in interior fish (Cell 5) have remained low, relative to inflow and outflow, and the ENR Project. However, similar to surface water, mosquitofish from STA-6 have also shown a trend of greater tissue mercury concentration at outflow compared to inflow (**Figure A7-9-8**, **Table A7-9-4**). Unlike water, this trend continued into the current reporting year. A two-way ANOVA evaluating site and date of collection found mercury concentrations differed by site ($df=1, 28$; $F=95.9$, $p < 0.001$).

Table A7-9-2. Concentrations of total mercury (THg) and methylmercury (MeHg) in surface waters collected quarterly from the STAs (units, ng/L).

STA	Quart	THg (ng/ L)			THg WQS [†]	MeHg (ng/ L)			% MeHg	
		Inflow	Remark	Outflow		Inflow	Remark	Outflow	Inflow	Outflow
			*	*			*	*		
STA 6	98-1	0.89		1.12	< WQS	(0.127) J4		(0.289) J4	NA	NA
	98-2	1.30 I		0.81 I	< WQS	(0.038) J4U		(0.038) J4U	NA	NA
	98-3	(1.45) ?		(1.47) ?	< WQS	(0.300) J A		(0.218) J	NA	NA
	98-4	1.18		1.51 A	< WQS	0.270		0.230 A	22.9	15.2
	99-1	(1.61) J3A		(0.67) J3I	< WQS	0.207		0.076 I	NA	NA
	99-2	1.32		0.59	< WQS	0.05 I A		0.04 I	3.79	6.78
	99-3	1.50 A		1.00	< WQS	0.31		0.07 A	20.67	7.00
	99-4	2.00		1.40	< WQS	0.15		0.15 A	7.5	10.71
	00-1	0.77		0.42 A	< WQS	0.13		0.12 A	16.89	28.57
STA 5 [‡]	00-1	2.2		1.65	< WQS	(0.049) J3		0.26	NA	15.76
STA1W	00-1	0.94 A		0.27	< WQS	0.055 I		0.05 I	5.85	18.52

* Data in parenthesis did not meet quality control checks; for qualifier definitions, see FDEP rule 62-160.

[†]Class III Water Quality Standard of 12 ng THg / L.

[‡] STA 5 has multiple inflows and outflows; reported value represents mean of valid data (unqualified).

Table A7-9-3. Percent change in concentration of THg and MeHg in surface water across STAs (i.e., outflow-inflow/inflow).

STA	Quarter	THg	MeHg	%MeHg
STA 6	98-1	26%	NA	NA
	98-2	-38%	NA	NA
	98-3	NA	NA	NA
	98-4	28%	-15%	-34%
	99-1	NA	-63%	NA
	99-2	-55%	-28%	79%
	99-3	-33%	-77%	-66%
	99-4	-30%	0%	43%
	00-1	-46%	-8%	69%
annual average		-41%	-28%	9%
Cumulative average		-21%	-32%	-5%
STA 5	00-1	-25%	NA	NA
annual average		-25%		NA
Cumulative average		-25%		NA
STA 1W	00-1	-71%	-9%	217%
annual average		-71%	-9%	217%
Cumulative average		-71%	-9%	217%

** Only valid (unqualified) data used in calculations; see Table 2 for raw data and qualifiers.

Table A7-9-4. Concentration of total mercury (THg) in mosquitofish composites collected semi-annually from STAs (units ng/g wet weight).

STA	Half-year*	Inflow fish (Mean \pm 1SE) [†]	Interior fish (Mean \pm 1SE) [†]	Outflow fish (Mean \pm 1SE) [†]	Percent change [‡]
STA 6	98-1	36	41	67	86%
	98-2	33	6	55	69%
	99-1	20	10	37	82%
	99-2	42	16 \pm 18	48	15%
	2000-1 [§]	NA	NA	NA	NA
	annual mean	31	13	42	49%
	cumulative mean	33	18	52	63%
STA 5	2000-1	38 \pm 1	67 \pm 19	61 \pm 24	61%
	annual mean	38	67	61	61%
	cumulative mean	38	67	61	61%
STA 1W	2000-1	33	21 \pm 14	35	7%
	annual mean	33	21	35	7%
	cumulative mean	33	21	35	7%

* Mosquitofish are collected semi-annually at inflow, interior and outflow sites.

[†] Standard error is reported where multiple composites are collected from location (e.g., multiple inflows or outflows, multiple cells); other values represent mean of five analyses of a single composite sample.

[‡] Percent change = outflow-inflow / inflow

[§] Semi-annual mosquitofish collected from STA 6 after April 30, 2000.

Table A7-9-5. Standardized (EHg3) and arithmetic mean concentration of total mercury (THg) in fillets from largemouth bass collected annually at STAs (ng/g, wet weight).

STA	Year	EHg3 \pm 95 th CI (mean \pm 1SD, n)			Percent change*	Consumption advisory exceeded [†]
		Inflow	Interior	Outflow		
STA 6 [‡]	1998	366 \pm 58 (265 \pm 149, 9)	NC (1) (726 \pm 194, 17)	629 \pm 72 (629 \pm 214, 20)	72%	Yes
	1999	368 \pm 36 (308 \pm 101, 20)	NC (2) (359 \pm 248, 3)	587 \pm 68 (498 \pm 185, 19)	60%	Yes

*Percent change across STA (i.e., outflow – inflow/inflow).

[†]Florida limited fish consumption advisory threshold is 500 ng/g in 3-yr-old bass.

[‡]Unable to collect 20 fish from each site.

NC – not calculated for: (1) insignificant slope or (2) if poor age distribution.

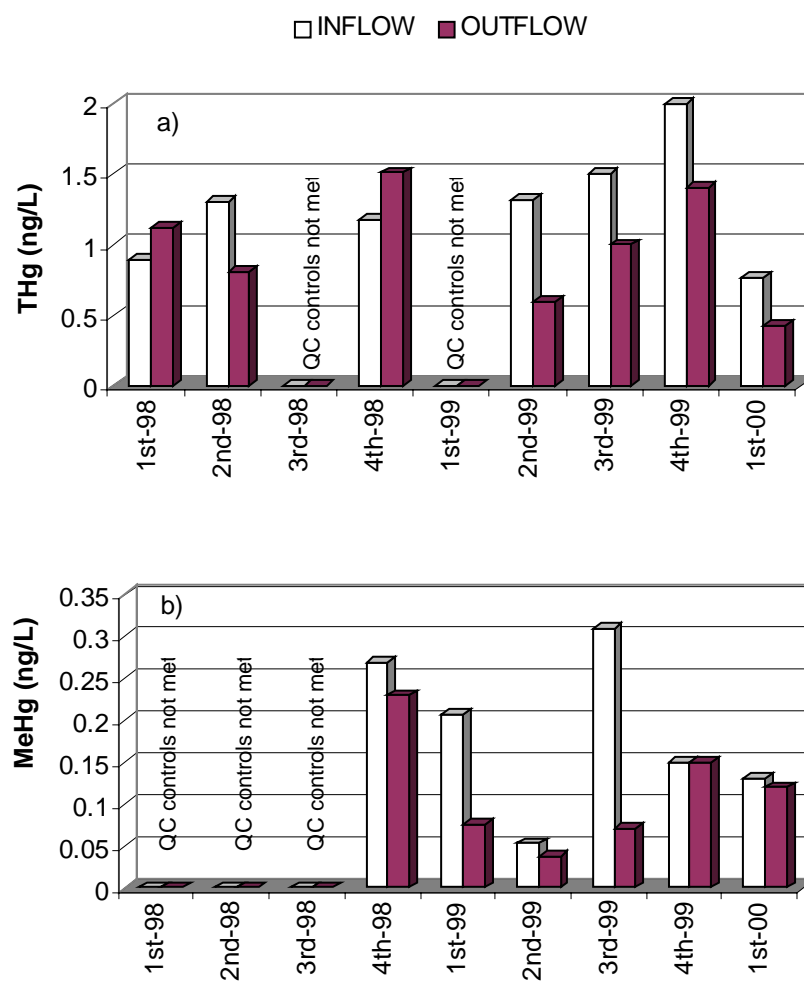


Figure A7-9-7. Surface water total mercury (a) and methylmercury (b) concentrations at STA-6. Samples (unfiltered) are collected quarterly.

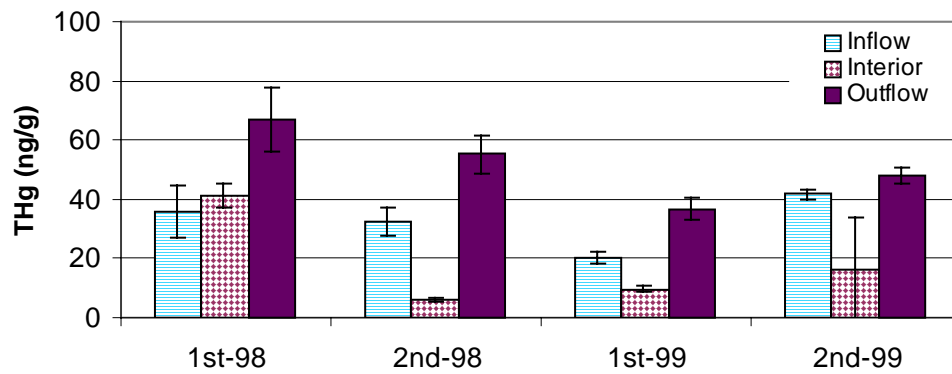


Figure A7-9-8. Total mercury concentrations in mosquitofish composite samples (n=100 individual fishes) collected semi-annually from STA-6. Error bars show analytical variability among five aliquots (i.e., ± 1 SD).

This analysis also showed a significant interaction between site and date of collection ($df=3, 28$; $F=7.89$, $p < 0.001$ [note, the F test for main effects was still valid because interaction was orderly, i.e., while magnitude of differences varied, the order of mean was the same]). A post-hoc pairwise comparison showed that in the second half of 1999, concentrations in outflow fish did not differ from inflow fish (Student-Newman-Keuls method, difference in means was 6.4 ng/g, $q = 2.53$, $p=0.084$; other pairwise comparisons were significant at $p < 0.05$). Percent difference across the STA in tissue mercury concentration in mosquitofish decreased from 86% in 1998 to 15% in second half of 1999, which also illustrates this increasing similarity between outflow and inflow mosquitofish.

Similar to mercury concentrations in sediment cores, tissue mercury concentration differed in mosquitofish collected from Cell 5 and Cell 3 in the second half of 1999 ($t=28.6$, $df=6$, $p < 0.001$). Sampling was expanded to include interior sites from both cells because of a concern by the District that the cells were behaving differently in terms of mercury biogeochemistry or bioaccumulation (further discussion of this issue can be found in **Appendix 7-13**).

Results from operational monitoring of mercury concentrations in largemouth bass from STA-6 over the two-year period are summarized in **Table A7-9-5** (values for individual fish are provided in **Table A7-9-A1** located at the end of this appendix). Unlike concentrations found in sediment, water, and mosquitofish that were comparable to the ENR Project, mercury concentrations in largemouth bass collected from STA-6 were substantially greater (up to 5x greater) than levels previously measured at the ENR Project (SFWMD, 1999b). However, concentrations of mercury in the STA-6 bass were comparable to levels observed in other areas of the Everglades (Lange et al., 1999; also see **Table A7-9-12**). Similar to mosquitofish, largemouth bass collected during both annual collections at STA-6 showed higher tissue mercury concentrations at the outflow as compared to inflow (**Table A7-9-5**). While this difference between inflow and outflow was shown by ANCOVA to be significant in 1998 ($df=1, 26$; $F=22.9$, $p < 0.0001$), because of an interaction between the effects of fish age and location on mercury concentration, ANCOVA could not be used to statistically evaluate spatial differences in 1999 (i.e., slopes were not parallel; $df=1, 35$; $F=4.65$; $p=0.04$). Notwithstanding the statistical limitations, bass collected at the outflow of STA-6 in 1999 clearly had substantially greater concentration of mercury than bass collected at the inflow. Notice that, similar to the mosquitofish, the degree of difference between inflow and outflow lessened in 1999 when levels of mercury in outflow fish decreased. The decline in mercury concentration in outflow bass from 1998 to 1999 was not significant, however (ANCOVA two-tailed test; $df=1, 36$; $F=3.73$, $p=0.12$).

Levels of mercury in bass from the interior of STA-6 must be interpreted carefully for a number of reasons. First, bass were collected from Cell 3 in 1998. In 1999, an effort was made to sample bass from both Cell 3 and Cell 5; however, only three bass were collected from Cell 5. The small sample size in 1999 and the lack of a significant age regression in 1998 (i.e., mercury concentration against bass age; $df=1, 16$; $F=0.0146$; $p=0.9$) did not allow for a valid test of between-cell differences on age-standardized basis. Nonetheless, mercury appeared to be at higher levels in interior bass from Cell 3 in 1998 compared to bass at the outflow, as well as interior fish collected from Cell 5 in 1999.

Bioaccumulation factors from water or sediments are gross over-simplifications of the real world situation. Nevertheless, these indices provide another means by which to

assess mercury-monitoring data. Bioaccumulation factor (BAF) is the ratio, in liters per kilogram, of THg concentration in fish flesh divided by concentration of MeHg (preferably dissolved) in the water column. Because of the variability in water column MeHg concentration, BAFs must be interpreted cautiously. The biota-sediment accumulation factor (BSAF) is a specialized form of the BAF that refers to the THg concentration in fish flesh divided by the concentration of MeHg (or THg) in sediments. The biomagnification factor (BMF; also known as the predator-prey factor or PPF) is the factor by which THg concentration in the organisms at one trophic level exceeds the concentration in the next lower trophic level.

BSAFs, BAFs and BMF observed at STA-6 (**Table A7-9-6**) were generally comparable to similar estimates reported for other areas in south Florida. For example, using median THg data from REMAP, a BSAF of about 0.6 was estimated for mosquitofish from canals in the Florida Everglades (USEPA, 1997). The BSAF for the largemouth bass (based on THg) was also comparable to ranges published in USEPA's "Mercury Study Report to Congress" (USEPA, 1997). However, as evident from **Table A7-9-6**, BAFs for fish at the outflow were much larger than at the inflow and were at the extreme range of reported values from other areas of the Everglades. The REMAP study (USEPA, 1998) reported BAFs for mosquitofish in the southern EAA to range from $0.7E+0.5$ to $1.2E+05$. The maximum observed BAF for largemouth bass from the ENR Project was $3.1E+05$ (T. Lange, pers. comm.). Likewise, the BMF was also larger at the outflow compared to the inflow. While the USEPA (1997) reports that BMFs between trophic level 3 and 4 fish range from 1 to 20 over the nation, values observed at STA-6, both at the inflow and outflow, were larger than BMFs observed just downstream in Everglades marshes (**Table A7-9-6**, also see **Appendix 7-13**).

Table 7-9A-6. Biota-sediment accumulation factors (BSAF), bioaccumulation factors (BAF), and biomagnification factors (BMF) observed at STA 6.

Fish	BSAF Interior*		BAF [†]		BMF [‡]	
	THg	MeHg	Inflow	Outflow	Inflow	Outflow
Mosquitofish	0.69	86	$1.96E+05$	$7.55E+05$	NA	NA
Largemouth bass	15.4	1,941	$1.76E+06$	$5.64E+06$	11.9	13.8

* BSAF was calculated as mean concentration in interior mosquitofish collected in September 1999 divided by mean concentration in sediment cores (wet wt.) collected in January 2000.

[†] BAF - MOSQ was calculated as mean of semi-annually collected mosquitofish divided by mean concentration in water over previous two quarters; BAF- bass was calculated as EHg(3) divided by mean concentration in water over previous four quarters.

[‡] BMF was calculated as concentration in bass divided by mean concentration in mosquitofish collected over previous year.

As previously stated, unlike other STAs, the timing, quantity, duration of inflows and backflows, and thus mean depth, hydraulic loading rate and hydraulic residence time (HDT) of STA-6 are controlled by USSC via its operation of G600. Operated this way since 1989, the area has repeatedly gone dry in the past. While the installation of the six outflow culverts (G-354 A-C for Cell 5 and G-393 A-C for Cell 3) by the District may reduce the frequency of drydowns, STA-6 will likely continue to dry out. For instance,

both Cell 3 and Cell 5 went dry for 2 months in March – May 1999 and again dry in March 2000 (for details see **Appendix 7-13** this report). As will be discussed in (**Appendix 7-8** this report), drydown and subsequent exposure and oxidation of sediments in the WCAs have been found to significantly influence mercury biogeochemistry and bioaccumulation. If similar conditions existed at STA-6 (e.g., redox potential, pH, and ratio of sulfate to sulfide), drydowns could account for the anomalous mercury results relative to the ENR Project, which did not go dry. However, the routine monitoring of STA soil pore water chemistry is not required in either the state of federal permits, so the quantification of these pre- and post-dryout influential factors has not occurred.

STA 5

While STA-5 met its THg and MeHg start-up criteria in the first biweekly sampling event, because outflow phosphorus concentrations remain substantially higher than inflow routine discharge has not begun as of April 30, 2000. Consequently, standing water conditions prevailed during water quality sampling (this caveat also applies to mosquitofish collection). Results from the first quarterly sampling of surface waters for mercury analysis at STA-5 are summarized in **Tables A7-9-2** and **A7-9-3**. As evident from the **Table A7-9-2**, the Florida Class III Water Quality Standard of 12 ng THg/L was not exceeded at either the inflow or the outflow. Concentrations of both THg and MeHg were within the typical range measured previously at the ENR Project (SFWMD, 1999b). Equally important, THg in surface water was at lower concentration at the outflow compared to the inflow. Concentration of THg decreased across the STA by 25% (**Table A7-9-3**). Because the analytical results for MeHg in inflow water failed to meet FDEP laboratory quality controls, concentration of MeHg cannot be evaluated in terms of inflow versus outflow (**Table A7-9-3**).

Results from operational monitoring of mercury concentrations in STA-5 mosquitofish are summarized in **Table A7-9-4**. Levels of mercury in STA-5 mosquitofish were comparable to concentrations observed in mosquitofish from the ENR Project (SFWMD, 1999b). However, tissue mercury concentrations in mosquitofish composites differed among collection sites at STA-5 (Kruskal-Wallis ANOVA on ranks; $H=21.9$, $df=2$, $p < 0.001$). Concentrations at the outflow were significantly greater than the inflow (Dunn's pairwise multiple comparison, $Q = 3.5$, $p < 0.05$). Interior mosquitofish did not differ from outflow fish in mercury concentration ($Q = 6.8$, $p > 0.05$), but contained higher levels than inflow fish ($Q = 4.1$, $p < 0.05$). When the analytical variability among replicate aliquots is considered, concentration did not differ between mosquitofish composites collected from the two interior cells ($q = 0.97$, $p > 0.05$).

STA-5 was not operational when the annual collection of largemouth bass took place (October-November 1999).

STA-1 West

Results from the first quarterly sampling of surface waters for mercury analysis at STA-1W are summarized in **Tables A7-9-2** and **A7-9-3**. It should be noted that at the time these samples were collected, construction of the second outflow pump, G310, was not yet complete; all outflow was through G251. As evident from the **Table A7-9-2**, the Florida Class III Water Quality Standard of 12 ng THg/L was not exceeded at either the

inflow or the outflow. Furthermore, concentrations of both THg and MeHg were within the typical range previously measured in this area when it was operated as the ENR Project (SFWMD, 1999b; note, the inflow station has moved from ENR002 to S5A). Equally important, both THg and MeHg in surface water was at lower concentration at the outflow compared to the inflow. Concentration of THg decreased across the STA by 71%, whereas MeHg decreased by only 9% (**Table A7-9-3**). The former value is typical of what was routinely achieved by the ENR Project prior to the expansion into STA-1W, but the MeHg removal efficiency is low when compared to the range of 65 –75% that was routinely achieved by the ENR Project.

Results from operational monitoring STA-1W mosquitofish are summarized in **Table A7-9-4**. Levels of mercury in STA-1W mosquitofish were relatively low and comparable to concentrations observed in mosquitofish previously collected from this area when it was operated as the ENR Project (SFWMD, 1999b). Although tissue mercury concentrations in mosquitofish composites from STA-1W differed among collection sites (ANOVA; $df=2, 22$; $F=6.2$ $p=0.007$), concentrations at the outflow did not differ from the inflow (Tukey Test; $q = 0.57$, $p=0.9$). Mercury was at lower concentration in interior mosquitofish (i.e., Cells 5, 4, 3 combined) than fish from both the inflow and outflow (Tukey Test; $q = 3.55$, $p=0.05$; $q = 4.25$, $p=0.02$; respectively). However, mosquitofish differed among cells. The level of mercury in fish from Cell 5 was greater than levels in fish from Cell 4 ($q = 3.6$, $p < 0.05$), but did not differ from Cell 3 ($q = 2.6$, $p > 0.05$); Cell 4 and Cell 3 did not differ ($q = 1.1$, $p > 0.05$).

STA-1W had not yet met its start-up criteria for MeHg and had not yet become operational during the period when the annual collection of largemouth bass took place (October-November 1999).

Downstream Receiving Water

Rainfall: National Atmospheric Deposition Program - Mercury Deposition Network

On a weekly basis, samples of bulk rainfall have been collected under the protocols of the National Atmospheric Deposition Program's Mercury Deposition Network at the ENR Project, at the Andytown substation owned by Florida Power and Light, and at the Baird Research Center Everglades National Park (for locations see **Figure A7-9-6**; for more information on MDN see <http://nadp.sws.uiuc.edu/mdn>). As evident from **Table A7-9-7**, volume-weighted average biweekly THg concentrations were highly variable both spatially and temporally. In general, results were consistent with the seasonal trends observed during the Florida Atmospheric Mercury Study (FAMS, Guentzel, 1997), with THg concentrations peaking in the summer (third calendar quarter) and declining to a minimum in winter (first calendar quarter). Guentzel (1997) reported THg concentrations in precipitation to be 2-3 times higher during the summer.

Table 7-9A-7. Biweekly mean bulk rainfall THg concentration data (ng/L) from the compliance sites of the National Atmospheric Deposition Program's Mercury Deposition Network in the reporting year ending April 30, 2000.

Week	ENR Project (FL34)	Andytown (FL04)	ENP (FL11)
17-18	12.19	15.23	15.09
19-20	19.60	17.87	17.37
21-22	32.38	9.17	13.20
23-24	6.43	10.26	16.21
25-26	13.76	7.38	4.21
27-28	20.64	8.31	11.59
29-30	31.56	27.96	15.41
31-32	33.21	11.58	26.70
33-34	19.83	21.11	9.98
35-36	18.37	14.61	14.09
37-38	12.29	12.72	14.98
39-40	9.41	11.14	6.78
41-42	1.75	3.20	2.57
43-44	5.59	6.54	14.30
45-46	35.08	4.47	22.21
47-48	6.79	3.81	21.14
49-50	15.31	12.35	6.02
51-52	16.50	4.19	6.52
2000 1-2	6.41	0.00	10.00
2000 3-4	9.94	7.46	8.78
2000 5-6	9.18	8.53	8.65
2000 7-8	0.00	16.69	14.25
2000 9-10	5.36	13.21	9.95
2000 11-12	5.41	10.55	13.69
1999 avg	10.94	10.96	11.62
2000 avg	10.39	10.89	11.65

Because rainfall volumes generally also increase during the summer by a factor of 2-3, Hg wet-deposition typically increases 5-8 fold during the wet season (**Figure A7-9-9**). Atmospheric wet-deposition of THg was lower in 1999 compared to 1998 (**Figure A7-9-9**); however, this between-year difference appears rainfall driven (i.e., due to less precipitation in 1999). In a recent assessment of the FAMs (1993-1996) and MDN (1996-1999) data sets, Pollman and Atkeson (2000) found no significant long-term temporal trend in wet deposition of Hg to south Florida.

Collectively, the results reported here for wet-deposition of THg in comparison with monitoring of surface water at Non-ECP Structures (following section) show that the major source of mercury to the Everglades is from the air. This is consistent with previous assessments by both FDEP (Atkeson, <http://www.dep.state.fl.us/labs/hg/flmercury.htm>) and U.S. EPA (USEPA, 1998). Dry deposition, which may exceed wet deposition by a factor of 2 (Keeler and Lindberg, **Appendix 7-5** this report), likely adds significantly to the overall atmospheric input. For detailed discussions of atmospheric deposition, the reader is referred to Appendices 7-5 and 7-6 of this report.

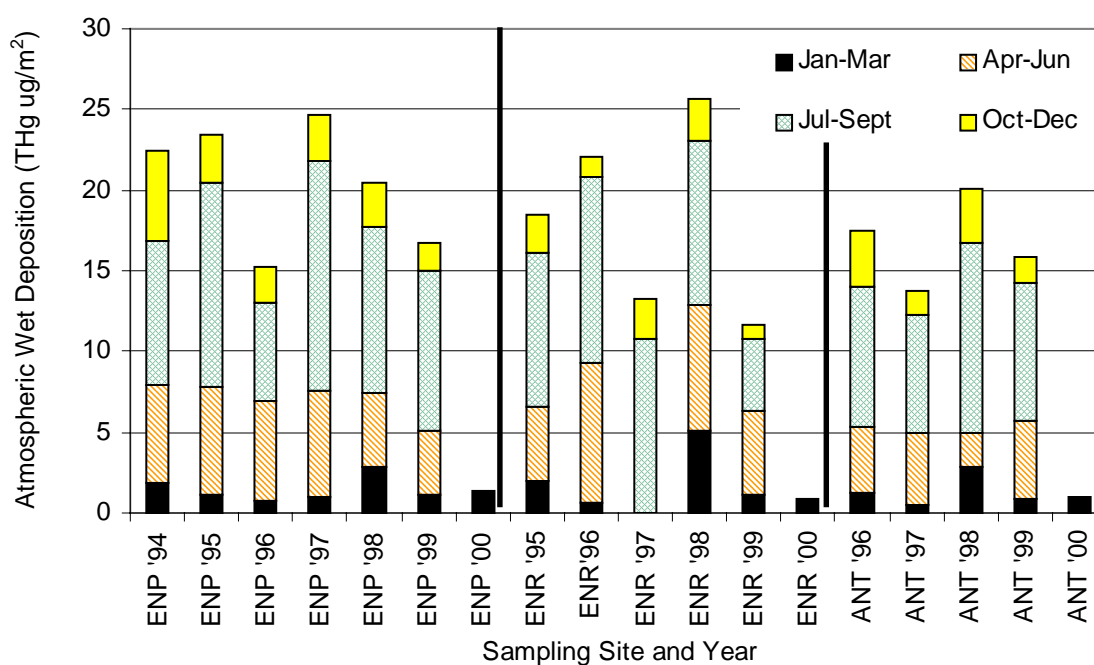


Figure A7-9-9.

Annual and quarterly atmospheric wet deposition as collected by FAMS and MDN. Note, first and second quarter ENR data are absent because MDN site did not become operational until 3rd quarter of 1997. Data presented based on calendar year.

Surface Water at Non-ECP Structures

Table A7-9-8 and **Figures A7-9-10** and **A7-9-11** summarize monitoring results of unfiltered THg and MeHg in surface water samples collected quarterly at Non-ECP structures (for map of locations see **Figure A7-9-5**). There are no baseline water concentration data generated by comparable analytical methods for any District structures prior to 1997. As in previous years, there were no exceedances of the Florida Class III Water Quality Standard for THg, 12 ng THg/L, at any of the structures monitored. The maximum THg concentration observed during the reporting year was 4.9 ng/L and occurred at S5A during the 1st quarter 2000. In general, THg concentrations were similar to or lower than previous years (denoted by large negative Mann-Kendal S values, **Table A7-9-9**). However THg concentrations in surface waters at one location, L28, which drains western watersheds including the C-139 Basin and Big Cypress Seminole Indian Reservation (L28 was surrogate for S190), exhibited a significant upward trend (n=8; S = 14, p=0.05).

The maximum MeHg concentration observed during the reporting year at a Non-ECP structure was 0.99 ng/L and occurred at L28 during the 1st quarter 2000. Note, currently, Florida has no WQS for MeHg. While MeHg at a few structures showed some indication of an increasing trend in concentration, particularly at L28 (denoted by large positive Mann-Kendal S values, **Table A7-9-9**), none of these trends were statistically significant (**Table A7-9-9**).

While L28 showed noteworthy temporal trends, it is important to note that median concentrations of THg and MeHg at this site were similar to or less than concentrations at other sites (period of record 5/1997 – 4/30/2000). THg concentrations differed among sites (Kruskal-Wallis ANOVA on ranks; df=9, H=18.6, p=0.029), with concentrations at S5A greater than S9, S32 and S334 (Dunn's multiple comparison procedure; p < 0.05); however, no other pairwise comparison was significant. A similar analysis found no significant among-structure differences in MeHg concentration (df=9, H=12.04, p=0.2).

Table A7-9-8. Concentrations of total mercury (THg) and methylmercury (MeHg) in Non-ECP structure surface waters (units, ng/L).

Structure	Quarter	THg			MeHg		% MeHg
		ng/L	remark **	WQS*	ng/L	remark **	
<u>L28</u>	99-2	0.67		<WQS	0.036	I	5%
	99-3	1.5		<WQS	0.11		7%
	99-4	1.4		<WQS	0.11		8%
	00-1	1.7	A	<WQS	0.99		58%
	Average last 4 qt.	1.32			0.312		24%
	cumulative avg.	1.16			0.19		16%
<u>S10C</u>	99-2	0.53		<WQS	0.028	I	5%
	99-3	1.5		<WQS	0.22		15%
	99-4	1.3		<WQS	0.11		8%
	00-1	0.05	U	<WQS	0.044	I	88%
	Average last 4 qt.	0.84			0.101		12%
	cumulative avg.	1.28			0.12		9%
<u>S12D</u>	99-2	0.97		<WQS	0.15		15%
	99-3	1.6	A	<WQS	0.16	A	10%
	99-4	1.6		<WQS	0.15		9%
	00-1	0.83		<WQS	0.13		16%
	Average last 4 qt.	1.25			0.148		12%
	cumulative avg.	1.27			0.15		11%
<u>S140</u>	99-2	0.34		<WQS	0.034	I	10%
	99-3	2.1		<WQS	0.18		9%
	99-4	1.3		<WQS	0.14		11%
	00-1	0.23	I	<WQS	0.057		25%
	Average last 4 qt.	0.99			0.103		10%
	cumulative avg.	1.35			0.15		11%
<u>S141</u>	99-2	1.4		<WQS	0.22		16%
	99-3	2.3		<WQS	0.26		11%
	99-4	0.65		<WQS	0.045		7%
	00-1	0.11	I	<WQS	0.035	I	32%
	Average last 4 qt.	1.12			0.14		13%
	cumulative avg.	1.21			0.15		12%
<u>S151</u>	99-2	NA		<WQS	NA		NA
	99-3	1.6		<WQS	0.14		9%
	99-4	0.77	A	<WQS	0.15		19%
	00-1	0.52		<WQS	0.092		18%
	Average last 4 qt.	0.96			0.127		13%
	cumulative avg.	1.25			0.12		10%
<u>S32</u>	99-2	0.48	A	<WQS	0.21		44%
	99-3	1.3		<WQS	0.13		10%
	99-4	0.74		<WQS	0.052	I	7%
	00-1	0.64		<WQS	0.044	I	7%
	Average last 4 qt.	0.79			0.109		14%
	cumulative avg.	0.86			0.11		12%

Table A7-9-8. Continued.

Structure	Quarter	THg ng/L	remark **	WQS*	MeHg ng/L	remark **	% MeHg
<u>S334</u>	99-2	0.85		<WQS	0.11		13%
	99-3	1.2		<WQS	0.18		15%
	99-4	0.083	I	<WQS	0.029	I	35%
	00-1	0.86		<WQS	0.095		11%
	Average last 4 qt.	0.75			0.104		14%
	cumulative avg.	0.95			0.12		12%
<u>S5A</u>	99-2	0.59		<WQS	0.079		13%
	99-3	3.1		<WQS	0.93		30%
	99-4	4.9		<WQS	0.058	I	1%
	00-1	1.5	A	<WQS	0.056	I	4%
	Average last 4 qt.	2.52			0.281		11%
	Cumulative avg.	2.66			0.243		9%
<u>S9</u>	99-2	0.63		<WQS	0.041	I	7%
	99-3	0.69		<WQS	0.039	I	6%
	99-4	1.6		<WQS	0.067		4%
	00-1	0.05	U	<WQS	0.03	I	60%
	Average last 4 qt.	0.74			0.044		6%
	Cumulative avg.	0.93			0.045		5%
	Ann. avg. 00-1	0.65	±0.59		0.16	±0.29	32%
	Ann. avg. 99-2	0.72	±0.32		0.10	±0.08	14%
	Ann. avg. 99-3	1.69	±0.67		0.24	±0.25	12%
	Ann. avg. 99-4	1.43	±1.31		0.09	±0.05	11%
	Cum. avg. 00-1	0.96	±0.50		0.11	±0.20	17%
	Cum. avg. 99-2	0.84	±0.45		0.11	±0.07	14%
	Cum. avg. 99-3	2.41	±1.13		0.22	±0.20	12%
	Cum. avg. 99-4	1.22	±0.94		0.12	±0.13	13%

*Class III Water Quality Standard of 12 ng THg/L

**Data in parentheses did not meet quality control checks; for qualifier definitions, see FDEP rule 62-160. Flagged values were not used in calculating averages.

NA – not available; sample was not collected because of fires.

Table A7-9-9. Temporal trend analysis of THg and MeHg concentrations in inflow surface water at Non-ECP structures using Mann-Kendall Test.

Analyte	Structure	N*	S†	Probability of S‡
THg	S5A	10	-5	negative S, no trend
	S10C	10	-15	negative S, no trend
	S141	10	-9	negative S, no trend
	S140	7	-11	negative S, accept Ho
	L28	8	14	p = 0.05; upward trend
	S9	9	-15	negative S, no trend
	S151	9	-2	negative S, no trend
	S32	8	-6	negative S, no trend
	S334	11	-15	negative S, no trend
	S12D	11	2	p = 0.47, no trend
MeHg	S5A	9	-6	negative S, no trend
	S10C	7	-1	negative S, no trend
	S141	8	-2	negative S, no trend
	S140	6	-7	negative S, no trend
	L28	9	13	p = 0.11, no trend
	S9	8	0	p = 0.55, no trend
	S151	9	2	p = 0.46, no trend
	S32	9	-6	negative S, no trend
	S334	9	-6	negative S, no trend
	S12D	9	1	p = 0.5, no trend
%MeHg	S5A	8	0	p = 0.55, no trend
	S10C	6	11	p = 0.028, upward trend
	S141	7	9	p = 0.12, no trend
	S140	5	2	p = 0.408, no trend
	L28	7	5	p = 0.281, no trend
	S9	7	3	p = 0.386, no trend
	S151	7	11	p = 0.068, no trend
	S32	7	-5	negative S, no trend
	S334	7	1	p = 0.5, no trend
	S12D	7	3	p = 0.386, no trend

* Only non-qualified data points were used in this analysis.

† Mann-Kendal statistic, if S is large negative number, measurements taken later in time tend to be smaller (Gilbert, 1987).

‡ One-tailed test: null hypothesis, Ho, of no trend against the alternative hypothesis, HA, of upward trend.

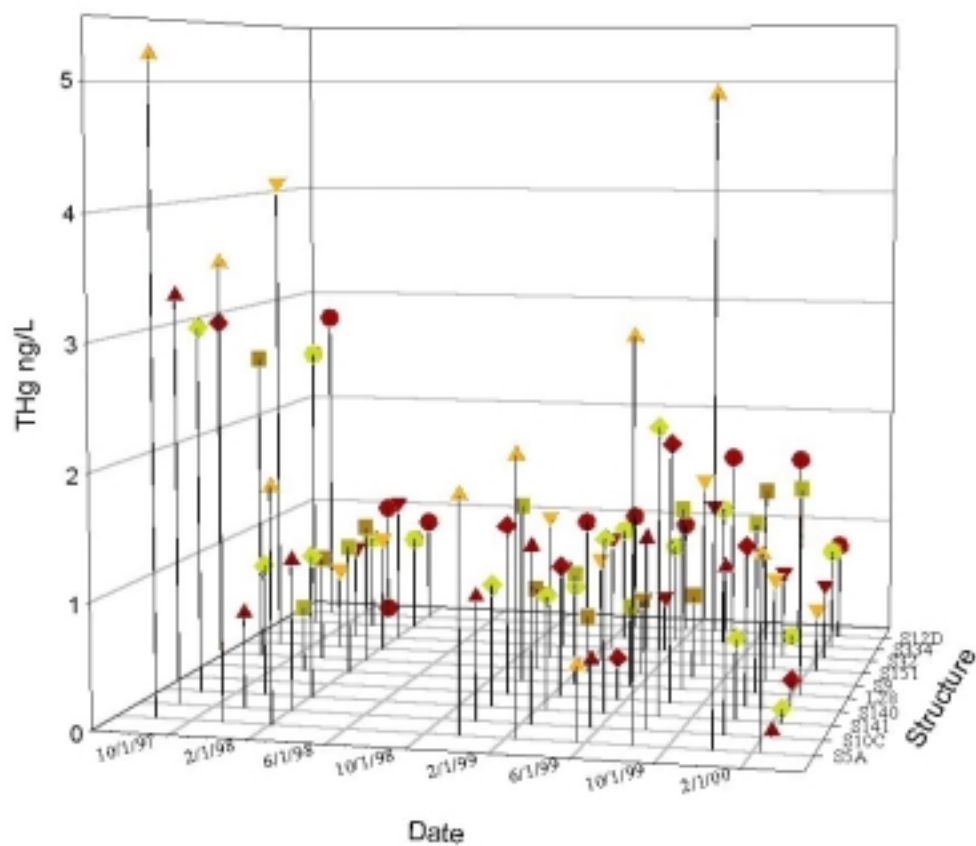


Figure A7-9-10. Total mercury concentrations in unfiltered surface waters at ten Non-ECP structures.

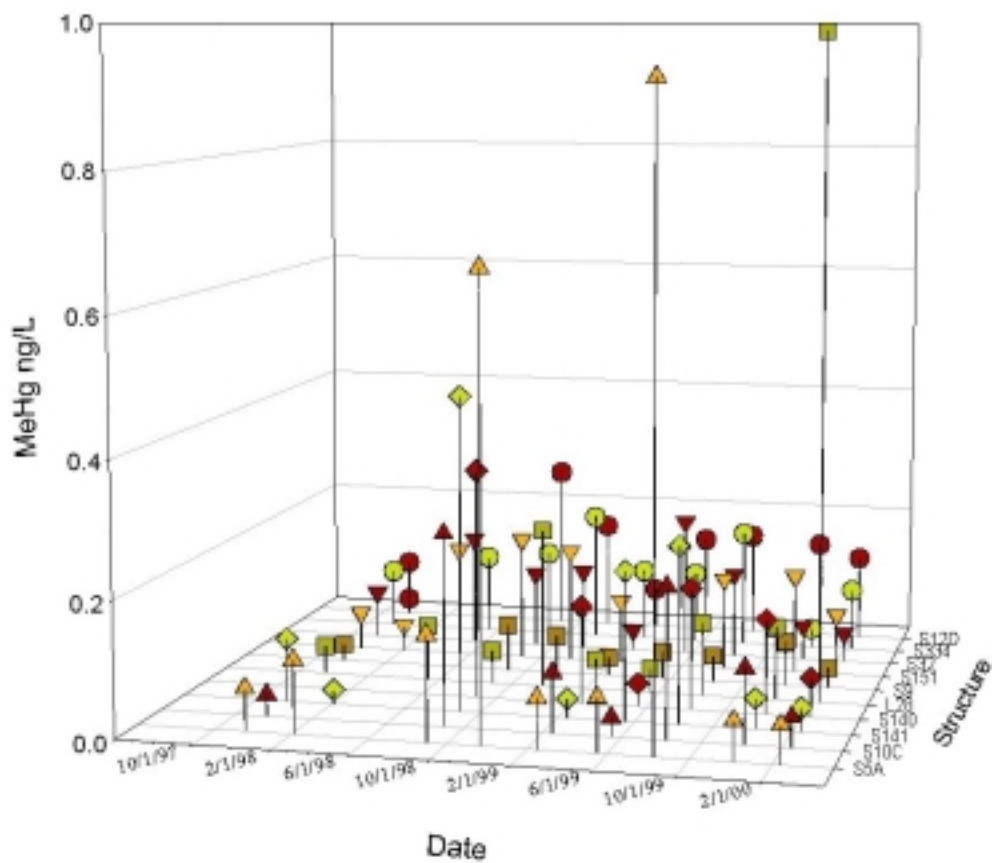


Figure A7-9-11. Methylmercury concentrations in unfiltered surface waters at ten Non-ECP structures.

Fish From ECP and Non-ECP Interior Marshes

Results from monitoring downstream interior marsh mosquitofish, sunfish and largemouth bass are summarized in **Tables A7-9-10** through **A7-9-12** (values for individual fish are provided in **Tables A7-9-A2** and **A7-9-A3** at the end of this document). Fish are collected from a total of 12 downstream interior marsh sites (**Figure A7-9-5**). Where fish could not be collected after a good faith effort, collection sites defaulted to nearby canals where fish were more plentiful and the same source water was being sampled. These default sites are depicted in **Figure A7-9-12**. Mercury levels in largemouth bass at three of these sites, LOX4 (WCA-1 GFC4), CA2U3 (WCA-2A U3), and CA3-15 (WCA-3A 15) were monitored by the FFWCC prior to initiation of the ECP (period of record extends back to 1993).

As will be discussed below, fishes collected in 1999 showed both spatial and temporal patterns in tissue mercury concentrations. In keeping with the primary objective of this monitoring program, the focus here will be on temporal changes in mercury concentration in fish tissues to assess possible adverse effects from the ECP and operation of the STAs. Nevertheless, spatial patterns of tissue mercury concentrations are important, particularly where there has been a variation from background conditions (i.e., pre-ECP conditions established by FFWCC). Therefore, spatial patterns will be reviewed in detail only where there has been change over time (i.e., interaction between treatment effects).

The average concentration of THg in mosquitofish collected from all marsh sites in 1999 was 196 ng/g (**Table A7-9-10**, for locations see **Figure A7-9-12**). This represents a 131% increase from the 1998 basin-wide average concentration. This between-year difference in mercury concentration in mosquitofish was significant (ANOVA of ln-transformed data; $df=1,75$; $F=231.9$; $p < 0.001$). Where mosquitofish were collected in both years, all sites showed significant increases in 1999 compared to 1998 (Student's t-test or rank sum test; $p < 0.01$). These increases ranged from 103% at LOX4 to 1032% at CA2F1 (**Table A7-9-10**). Despite the large increase, mercury concentrations in mosquitofish from CA2F1 remained low relative to other sites (**Figure A7-9-13**). In contrast, in 1999, CA2U3 mosquitofish, which in the past have contained low to moderate levels of mercury, resembled mosquitofish from the mercury "hot spot," CA3A15 (**Table A7-9-10**, **Figure A7-9-13**). Researchers with the U.S. Geological Survey (USGS) also report higher concentrations of mercury in WCA-2A mosquitofish collected in October 1999 (**Appendix 7-8**, this report).

Sunfish also exhibited interannual differences in tissue mercury concentration, but the magnitude of the between-year difference was smaller and the direction of change was variable among locations. Percent change in mercury concentration from 1998 to 1999 ranged from a 46% decrease in THg concentration at CA3F1 (L28 canal, alternate site for Non-ECP north) to an 86% increase in concentration at L67F1 (alternate site for ENP P33; **Table A7-9-11**, **Figure A7-9-14**).

Table A7-9-10. Concentration of total mercury (THg) in mosquitofish composites (units ng/g wet weight) collected from downstream sites. Value represents mean of 3-5 analyses.

Location	Lat.	Long.	THg (ng/g)		Between-yr. change (%)*	Cum. average
			1998	1999		
LOX3	26 35.750'N	80 21.330'W	112	NA		112
LOX4	26 27.750'N	80 17.950'W	77	156	103%	116
CA2 F1	26 21.58'N	80 22.23'W	6	74	1032%	40
CA2F1 Alt. (L39F1)	26 22.28'N	80 21.090'W	NA	118		118
CA27 (Marsh)	26 22.07'N	80 30.67'W	116	NA		116
CA27 Alt. (L38F1)	26 20.09'N	80 32.15'W	NA	282		282
Holey Land (North canal)	26 25.96'N	80 41.355'W	32	117	267%	74
Rotenberger Alt. (RotenF1)	26 19.99'N	80 48.928'W	NA	242		242
CA2U3	26 17.25'N	80 24.68'W	53	284	433%	169
CA33	26 17.97'N	80 37.89'W	NA	NA		
CA33 Alt (L5F1)	26 20.00'N	80 37.68'W	121†	222	84%	171
CA35	NA	NA	188	NA		188
Non-ECP North (CA3F1; end of L-28)	26 05.502'N	80 49.192'W	29	128	344%	78
CA315	26 00.305'N	80 38.927'W	110	284	157%	197
Non ECP South (CA3F2)	25 48.748'N	80 47.629'W	70	178	155%	124
P33	25 37.54'N	80 37.683'W	105	224	114%	164
P33 Alt. (L67F1)	25 37.54'N	80 40.366'W	NA	242		242
annual mean			84	196	131%	152

* All between-year differences were significant at $p < 0.01$.

† Value revised from 2000 Report; original value of 111.4 ng/g was incorrect mean of the five analyses.

NA = data not available due to the absence of fish at the site.

Table A7-9-11. Mean concentration (± 1 SD; ng/ g wet weight) of total mercury (THg) in sunfish (*Lepomis spp.*) collected from interior marsh within downstream waters.

Target location	Sampling Location	Lat. N	Long.W	Mean THg ng/g (± 1 SD, n)		Between-yr. change (%)	Cum. average
				1998	1999		
WCA1-LOX3	LOX4	26°27.75	80°17.95	221 (± 60 , 3)	144 (± 66 , 14)	-35%	183
WCA-2A F1	L39F1	26°21.580	80°22.230	102 (± 67 , 28)	75 (± 48 , 20)	-26%	88
WCA-2A 2-7	L38F1	26°20.092	80°32.149	151 (± 78 , 20)	104 (± 38 , 20)	-31%*	128
Holey Land	Holey Land	26°26.120	80°41.540	38 (± 24 , 20)	40 (± 13 , 20)	5%	39
Rotenberger†				NA			
WCA-2A U3	CA2U3	26°17.250	80°26.680	106 (± 66 , 21)	156 (± 61 , 19)	47%*	131
WCA-3A 3	L5F1	26°20.004	80°37.683	72 (± 44 , 20)	88 (± 55 , 19)	23%	80
WCA-3A 5†				NA			
Non-ECP North	CA3F1	26°05.502	80°49.192	185** (± 166 , 20)	117 (± 77 , 20)	-37%	151
WCA-3A 15	CA315	26°00.305	80°38.927	375 (± 234 , 20)	371 (± 182 , 20)	-1%	373
Non-ECP South	CA3F2	25°48.748	80°47.629	263 (± 171 , 20)	213 (± 124 , 20)	-19%	238
ENP P33 Marsh	L67F1	25°37.540	80°40.366	350 (± 167 , 11)	651 (± 672 , 20)	86%*	548
ENP P33 Marsh	P33 Marsh	25°37.541	80°37.683	646 (± 203 , 9)	447 (± 132 , 3)	-31%	547

* Significant between-year difference in concentrations; $p < 0.05$.

† Unable to collect 20 fish from each site; NA – not available.

** Value revised from 2000 Report; original value of 216 ng/g was incorrect mean.

NA = data not available due to the absence of fish at the site.

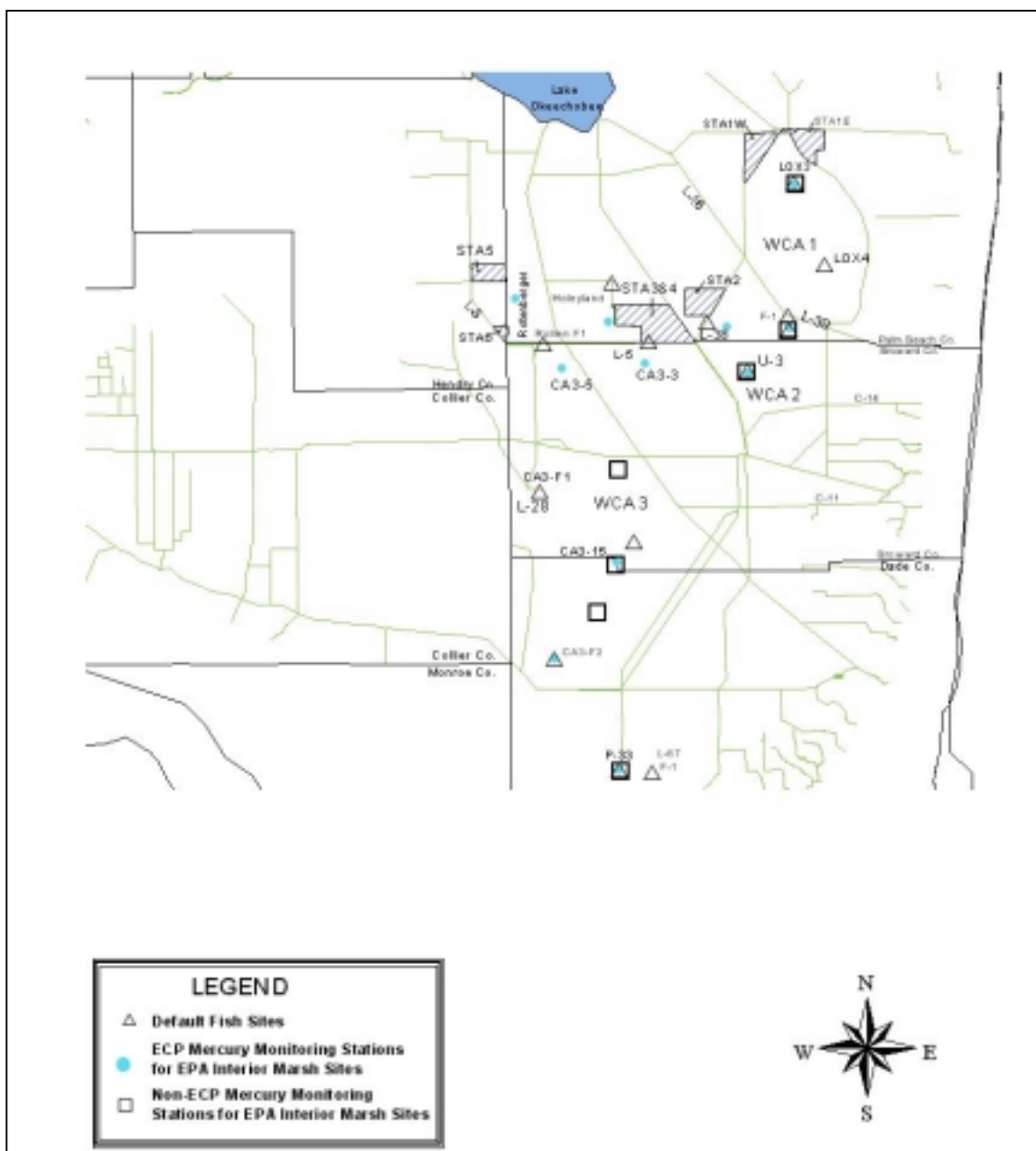


Figure A7-9-12. Default collection sites for large-bodied fish.

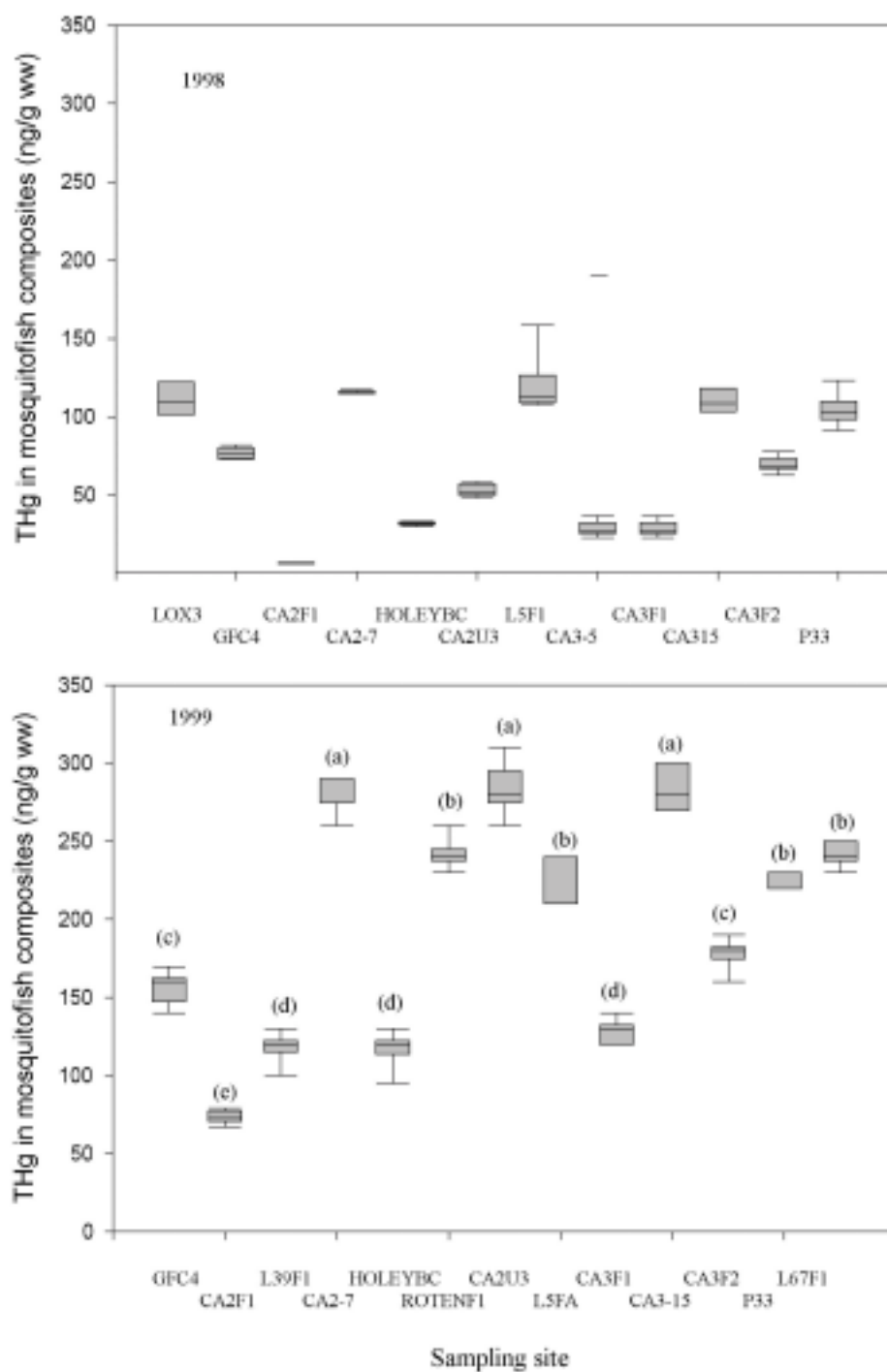


Figure A7-9-13. Boxplots of mercury concentrations in mosquitofish (*Gambusia sp.*) collected at ECP and Non-ECP sites in 1998 and 1999. Not all sites sampled in both years (for details, see Table A7-9-10). Sites in 1999 with similar letter designations did not differ significantly.

Table A7-9-12. Standardized (EHg3) and arithmetic mean concentrations of total mercury (THg) in largemouth bass fillets (ng/g wet weight) collected from ECP and Non-ECP interior marsh sites.

Target Location	Sampling Location	Lat. N	Long. W	EHg3 \pm 95 th CI (mean \pm 1SD, n) ng/g wet		Consumption advisory exceeded*	Cum. mean
				1998	1999		
CA1-LOX3	LOX4	26°27.75	80°17.95	671 \pm 94 (517 \pm 298, 21)	405 \pm 66 (292 \pm 122, 19)	No	405
CA2-F1	L39F1			NC (2) (NA, 0)	312 \pm 95 (337 \pm 231, 11)	No	
CA2-7	L38F1	26°20.092	80°32.149	445 \pm 197 (677 \pm 358, 20)	450 \pm 83 (457 \pm 263, 20)	No	567
Holeyland	HOLYBC	26°26.120	80°41.540	281 \pm 58 (318 \pm 196, 20)	256 \pm 135 (481 \pm 258, 19)	No	400
Rotenberger†				NC (2) (NA, 0)	NC (2) (NA, 0)		
CA2-U3	CA2U3	26°17.250	80°26.68	521 \pm 76 (379 \pm 209, 18)	668 \pm 74 (568 \pm 256, 21)	Yes	474
CA3-3	L5F1	26°20.004	80°37.683	353 \pm 82 (446 \pm 130, 20)	NC (1) (414 \pm 186, 20)	No	430
CA3-5†				NC (2) (NA, 0)	NC (2) (NA, 0)		
Non-ECP North	CA3F1	26°05.502	80°49.192	NC (1) ‡(765 \pm 417, 20)	586 \pm 92 (556 \pm 321, 20)	Yes	661
CA3-15	CA3-15	26°00.305	80°38.927	NC (2) ‡(1272 \pm 1226, 8)	1013 \pm 155 (715 \pm 450, 21)	Yes	994
Non-ECP South	CA3F2	25°48.748	80°47.629	NC (1) (986 \pm 1271, 5)	NC (2) (735 \pm 516, 2)	Yes	861
ENP-P33	L67F1	25°37.540	80°40.366	1170 \pm 285 (1152 \pm 718, 20)	NC (1) (1041 \pm 424, 15)	Yes	1097

* Florida limited fish consumption advisory threshold is 500 ng/g in 3-yr-old bass.

† Unable to collect fish from site.

‡ Mean revised from previous report to reflect flagged value or mislabeled fish.

NC - not calculated for: (1) insignificant slope or (2) if poor age distribution. NA - not available.

As discussed previously, attempts were made to use analysis of covariance (ANCOVA) to evaluate patterns of mercury concentrations in sunfish, *Lepomis spp.*, using size as a covariate. However, use of ANCOVA was inappropriate because size-concentration relationships were inconsistent among sites (i.e., slopes were either not significant or were not parallel based on fish total length or weight). The lack of a strong concentration-size relationship likely resulted from interspecies differences (i.e., among the different *Lepomis* species) in growth and bioaccumulation factors. Sunfish species was found to be a significant factor in tissue mercury concentration (ANOVA on ln-transformed data, $df=3$, 378; $F=54.2$, $p < 0.001$), with mercury concentrations in *L. gulosus* (warmouth) $>$ *L. punctatus* (spotted sunfish) $>$ *L. macrochirus* (bluegill) $>$ *L. microlophus* (reardear sunfish) (Tukey test multiple comparison procedure, $p < 0.01$). These interspecies differences in tissue mercury concentration were not a function of size differences; weight of *L. gulosus* = *L. microlophus* $>$ *L. macrochirus* $>$ *L. punctatus* (Dunn's multiple comparison procedure, $p < 0.05$). It is important to note that there was no significant interaction between year and species ($df=3$, 374; $F=1.8$; $p=0.14$). Nevertheless, an attempt to derive size-concentration regressions for individual species for use in ANCOVA also failed. For example, bluegill (*Lepomis macrochirus*), the most commonly collected species, showed a significant size-concentration relationship (i.e., non-zero slope) only at the L67F1 site.

Because sunfish tissue mercury concentrations also failed assumptions of normality (raw data) and equal variance (transformed data), year and location effects could not be tested using a two-way ANOVA. Instead, between-year differences were assessed at each location using a Student's t-test. Of the nine sites where sample size was sufficient in both years for a valid test ($n > 9$, LOX4 and P33 not tested), only L38F1, CA2U3 and L67F1 were found to have significant between-year differences in mercury concentrations (ln-transformed). Sunfish at L38F1 (WCA-2A) had lower mercury concentration in 1999 compared to 1998 ($df=38$, $t=-2.2$, $p=0.04$). Conversely, sunfish both at CA2U3 and L67F1 contained significantly greater tissues concentrations of mercury in 1999 compared to 1998 ($df=37$, $t=-3.0$, $p=0.005$; $df=29$, $t=-2.14$, $p=0.04$; respectively). In particular, fish collected at L67F1 in 1999 contained some of the highest concentrations of mercury ever observed in Everglades *Lepomis*. A 45 gm bluegill (137 mm), for example, was found to have 3300 ng THg /g (3.3 ppm), which is almost 5x greater than the next highest concentration previously reported for this species. It is noteworthy that while CA2U3 mosquitofish resembled CA3A15 mosquitofish (i.e., the mercury "hot spot"), CA2U3 sunfish contained less than ½ the THg concentration found in CA3A15 sunfish (**Table A7-9-11**).

It is also important to note that there were no dramatic shifts in the species of *Lepomis* collected at L38F1, CA2U3 or L67F1 from 1998 to 1999. Furthermore, while sunfish from L67F1 were smaller in 1999 compared to 1998 ($df=28$, $t=2.4$, $p=0.02$), weights did not differ between years at L38F1 and CA2U3 ($df=38$, $t=-0.06$, $p=0.95$ and $df=37$, $t=-1.64$, $p=0.1$, respectively). Therefore, the observed between-year difference in mercury concentration was not attributable to variability in fish species or size.

Similar to the lower trophic level fish, largemouth bass exhibited significant patterns in tissue mercury concentrations over both space and time. Between-year differences in standardized age(3) expected mercury concentration (EHg3) ranged from a 40% decrease

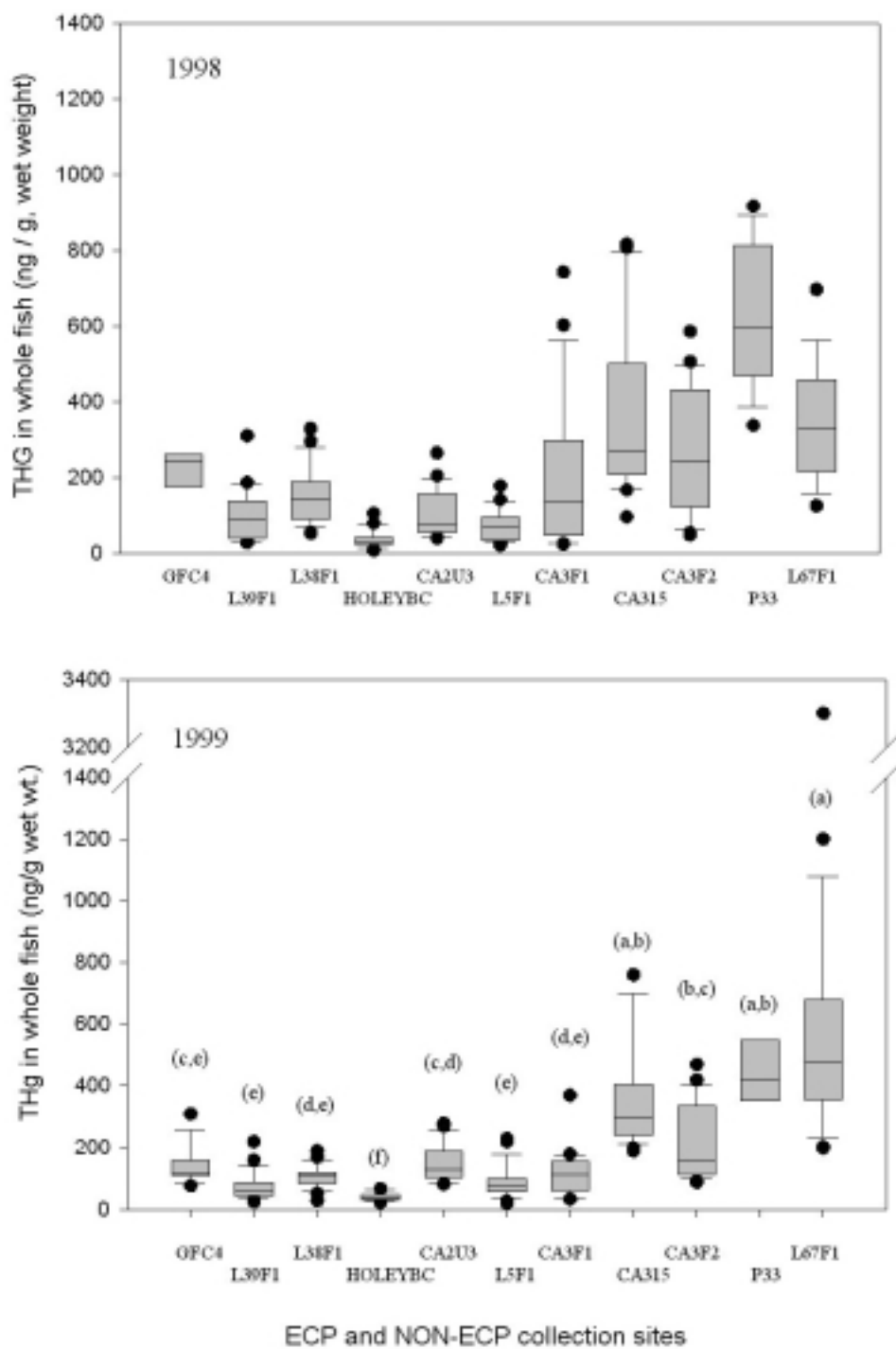


Figure A7-9-14. Boxplots of THg concentration in whole sunfish (*Lepomis spp.*) collected at ECP and Non-ECP sites in 1998 and 1999. Outliers that lie outside the 10th and 90th percentile shown as filled circles. Sites in 1999 with similar letter designations did not differ significantly in fish mercury concentration.

in concentration at LOX4 (alternate site for WCA-1: LOX3) to a 28% increase at CA2U3 (Table A7-9-12, Figure A7-9-15). This between-year difference in tissue mercury concentrations in bass from CA2U3 was statistically significant (ANCOVA, $df=1, 36$; $F=16.43$; $p=0.0003$). Between-year difference in mercury concentration at LOX4 could not be assessed using ANCOVA because of an observed interaction between age and year (i.e., regression lines were not parallel; $df=1, 36$; $F=16.51$, $p=0.0003$).

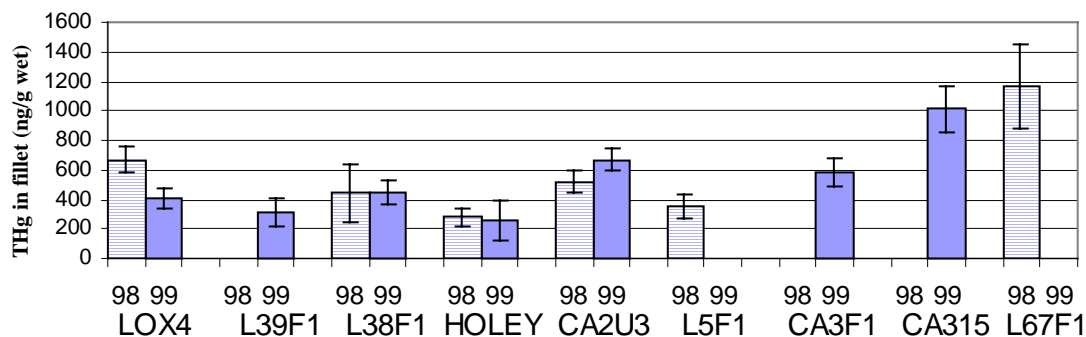


Figure A7-9-15. Standardized age(3) expected mercury concentration (EHg3) in largemouth bass (*Micropterus salmoides*) collected at ECP and Non-ECP sites in 1998 and 1999.

The CA2U3 site is particularly noteworthy because it showed a significant increase in mercury concentration over last year in all three fish taxa (i.e., at each trophic level). This is not to say that other sites did not show consistent relationships at different trophic levels. For example, as already mentioned, largemouth bass from LOX4 showed a 40% decrease in mercury. This was consistent with a 35% reduction in mercury in sunfish at that site. Similarly, concentration of mercury in bass from the Holey Land differed only slightly between years, which was consistent with the stable concentrations observed in sunfish. On the other hand, as discussed above with regard to mercury levels in mosquitofish and sunfish, statistically significant increases in mercury in one trophic level did not allow one to infer observable increases in the next trophic level with confidence. For example, L67F1, the site other than CA2U3 found to have significantly greater concentrations of mercury in 1999 sunfish, did not appear to show greater mercury concentration in 1999 bass.

However, this apparent discrepancy may be reconciled by closer inspection of the data. The regression of mercury concentration on age of largemouth bass from L67F1 was not significant (i.e., slope did not differ from 0; $df=1,12$; $F=2.506$, $p=0.14$). This was due primarily to the high concentrations observed in year-1 fish relative to other age cohorts collected at this site (Figure A7-9-16). Where Year-1 fish were collected, they tended to show higher concentrations relative to older cohorts collected in the same year (e.g., L38F1, CA2U3, CA315, L67F1; Figure A7-9-16). This observation highlights an important point. Because largemouth bass are a relatively long-lived fish (oldest fish collected in 1999 was estimated to be 8.8 years), between-year differences in arithmetic mean concentrations can be confounded by the age distribution of the collected fish. It is

for this reason that concentrations are standardized to a 3-year-old fish. The selected age (and size) is appropriate where the focus is on human health concerns from ingesting harvestable-size fish (i.e., 3-year-old fish). However, standardization to age(3) also tends to dampen out the effects of short-term changes. For example, while standardized age(3) mercury concentrations in bass at CA2U3 showed a 28% increase in mercury in 1999 over 1998, the arithmetic mean concentration in 2-year-old bass increased by 57% over concentrations in a similar aged cohort in 1998 (t-test, $df=17$, $t=-2.3$, $p=0.032$). Not surprisingly, the increase was even more dramatic in the Year-1 cohort, which showed a 169% increase in mercury over Year-1 fish collected in 1998 (t-test, $df=6$, $t=-12.7$, $p<0.001$). Similar evaluations could not be done for other sites due to the small numbers of Year-1 fish collected in 1998. This suggests that there was a change in one or more factors governing MeHg bioaccumulation that had its greatest effect on the youngest fish, from which one could infer that the effect on MeHg bioaccumulation occurred in 1999. If real, these observed increases could have a substantial impact on the exposures of young-of-the-year wading birds to MeHg in the Everglades ecosystem. A possible explanation of this apparent increase in MeHg bioaccumulation is taken up in **Appendix 7-8**.

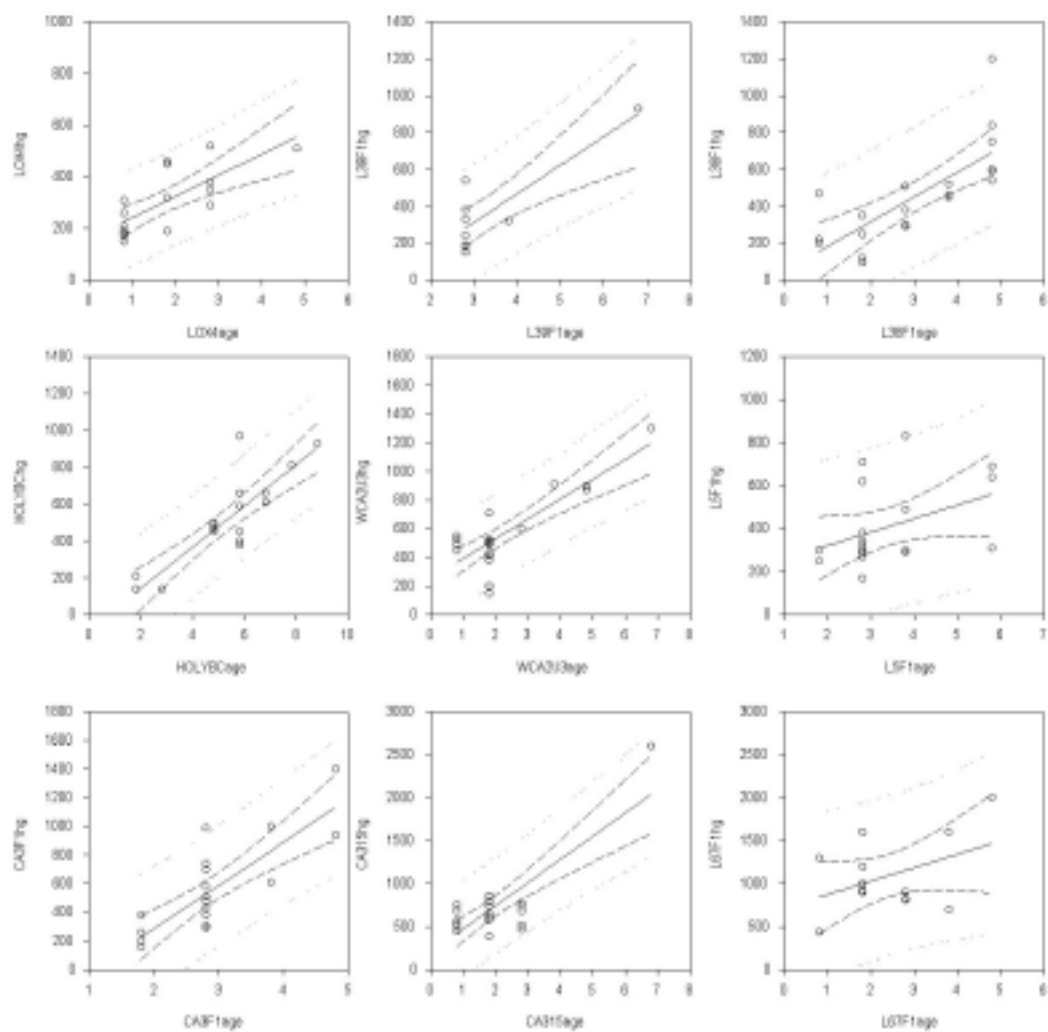


Figure A7-9-16. Relationship between age and THg concentration (ng/g wet) in largemouth bass collected from Everglades sites in 1999.

BSAFs observed at downstream sites (i.e., at fish collection sites where sediment data was available) are summarized in **Table A7-9-13**. With the exception of the BSAF-THg for mosquitofish at CA2F1, all BSAFs declined in 1999, some substantially. This change in BSAF appears to be a result of an increase in THg concentrations in the sediments; however, the significance of the observed differences must be interpreted cautiously due to possible methodological/analytical differences between the two sources of sediment-THg data. While the 1998 BSAF for CA2F1 mosquitofish (THg) was comparable to published values (USEPA, 1997), other BSAFs appear elevated. THg data presented by Sorenson et al. (1990) yield a BSAF (dry weight basis) of approximately 10.1 for northern pike. Data presented by Wren and MacCrimmon (1986) allow BSAFs to be calculated for two Ontario lakes. BSAFs (dry weight basis) were very similar in both lakes, ranging from approximately 5.1 (clams) to 24.0 (northern pike) in the less contaminated of the two lakes, and 3.4 (clams) to 27.1 (pike) in the other system. In the present study, BSAFs differed greatly among locations in 1998 (THg) and during both years when based on MeHg concentrations in sediments. Interestingly, in 1998, BSAFs for both THg and MeHg at CA2U3 were 2x and 10x times higher for sunfish and largemouth bass compared to CA2U3 mosquitofish, respectively.

Table A7-9A13. Biota-sediment accumulation factors (BSAF, based on wet wt.) observed at downstream Everglades marsh sites.

	THg		MeHg	
	1998	1999	1998	1999
Mosquitofish				
CA2F1	0.5	7.4	140	100
CA2U3	11	10.7	2,038	389
CA315	108	7.7	5,789	2,705
Sunfish				
CA2U3	22	6	4,077	214
CA315	368	10	19,737	3,533
Largemouth bass EHg(3)				
CA2U3	109	25	20,038	915
CA315	NA	28	NA	9,648

1998 sediment data (top 4 cm, wet wt.) from June 1998 (C. Gilmour, pers. comm.)

1999 sediment data (top 5 cm, wet wt.) from July 1999 (D. Krabbenhoft, pers. comm.)

Fish data from annual collections made from September-November.

BAFs for mosquitofish increased in 1999, particularly at CA2F1 where the BAF increased by an order of magnitude (**Table A7-9-14**). Nevertheless, BAFs for mosquitofish were still within the range reported from REMAP ($1.7\text{E}+05$ – $2.7\text{E}+05$; USEPA, 1998). In 1999, BAFs decreased at CA2U3 for both sunfish and bass, presumably owing to an increase in MeHg in the water column. The BAFs for the CA2U3 largemouth bass were comparable to values reported for other areas of the Everglades (T. Lange, pers. comm.).

Table A7-9-14. Bioaccumulation factors (BAF) observed at downstream interior marsh sites.

	1998	1999
Mosquitofish		
CA2F1	1.83E+04	1.74E+05
CA2U3	2.07E+05	2.37E+05
Sunfish		
CA2U3	4.13E+05	1.30E+05
Largemouth bass EHg(3)		
CA2U3	2.03E+06	5.57E+05

1998 based on mean concentration of MeHg in duplicate unfiltered samples collected on 8/28/98.

1999 based on mean concentration of MeHg in filtered samples collected 6/28/99 and 8/23/99.

As evident from **Table A7-9-15**, BMFs were highly variable at downstream interior marsh sites ranging from 0.3 to 7 for mosquitofish - sunfish, 2 to 9 for mosquitofish - bass and, 3 to 7 for sunfish - bass. Several different patterns were discernable in the BMF data set. First, BMFs for mosquitofish - sunfish generally increased from north to south in both 1998 and 1999. Second, owing to the previously discussed increases in THg in mosquitofish, BMFs in mosquitofish - sunfish decreased substantially at all sites in 1999 compared to 1998. Where a BMF was less than 1, mosquitofish contained greater THg concentrations than sunfish. For similar reasons, BMFs for mosquitofish - largemouth bass EHg(3) decreased in 1999. Conversely, the BMFs for sunfish - largemouth bass did not differ substantially between years. As discussed previously, BMFs between trophic level 3 and 4 fish range from 1 to 20, but the “national average” is 4.9 (USEPA, 1997).

Table A7-9-15. Biomagnification factors (BMF) observed at downstream interior marsh sites.

Location	Mosquitofish to Sunfish		Mosquitofish to Bass EHg(3)		Sunfish to Bass EHg(3)	
	1998	1999	1998	1999	1998	1999
LOX4	3	0.9	9	3	3	3
CA2 F1	NA	NA	NA	NA	NA	NA
L39F1	NA	0.6	NA	3	NA	4
CA27	NA	NA	NA	NA	NA	NA
L38F1	NA	0.4	NA	2	3	4
Holey Land	1	0.3	9	2	7	6
RotenF1	NA	NA	NA	NA	NA	NA
CA2U3	2	0.5	10	2	5	4
CA33	NA	NA	NA	NA	NA	NA
L5F1	1	0.4	3	NA	5	NA
CA35	NA	NA	NA	NA	NA	NA
CA3F1	7	0.9	NA	5	NA	5
CA315	3	1.3	NA	4	NA	3
CA3F2	4	1.2	NA	NA	NA	NA
P33	6	2.0	NA	NA	NA	NA
L67F1	NA	2.7	NA	NA	3	NA
Mean	3	1	8	3	4	4

The environmental significance of this apparent between-year increase in bioavailable MeHg and fish tissue concentration can only be determined with more time. As stated in the previous report (Rumbold and Rawlik 2000), short-term temporal trends must be interpreted cautiously because long-term monitoring by the FFWCC has shown concentrations of THg in bass at several sites to decrease monotonically over several years, then increase (e.g., ENP P33, etc). When viewed in context with previously observed interannual variations in mercury concentration in bass (**Figure A7-9-17**), the increase observed in 1999 appears to be within the temporal variation attributable to natural processes. However, an increase would be a departure from the trend that has been developing over the last five years.

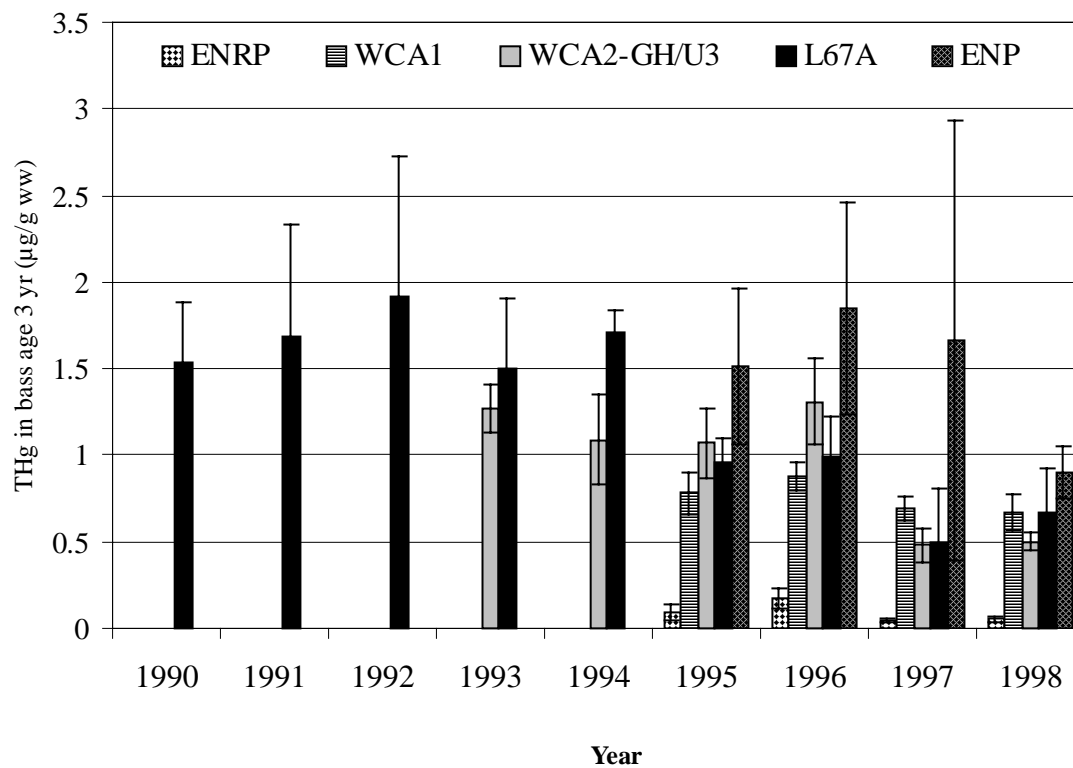


Figure A7-9-17. Total mercury in largemouth bass over time (data from Lange et al. 1999).

Lange et al. (1999; 2000) report that standardized age (3) mercury concentrations declined at several sites, including sites in WCA-3A and WCA-2A, since 1996, with the most significant decreases occurring between 1996 and 1997; subsequent to 1997 concentrations appear to have leveled off (**Figure A7-9-17**).

Levels of mercury in fish tissues can also be put into perspective and evaluated with regard to mercury risk to wildlife. The U.S. Fish and Wildlife Service (USFWS) has proposed a predator protection criterion of 100 ng/g THg in prey species (Eisler, 1987). More recently, in its “Mercury Study Report to Congress”, USEPA proposed 77 ng/g and 346 ng/g for trophic level (TL) 3 and 4 fish, respectively, for the protection of piscivorous avian and mammalian wildlife (USEPA, 1997). With the exception of mosquitofish from CA2F1 (**Table A7-9-10**), all mosquitofish, which are considered to be at TL 2-3 depending on age (Loftus et al., 1998), exceeded both the USFWS and USEPA criteria in 1999. Likewise, based on mean concentrations (**Table A7-9-11**), sunfish, which are at TL 3 (*L. gulosus* at TL 4; Loftus et al., 1998), at most sites exceeded both predator protection criteria in 1999 (sunfish from L39F1, Holey Land and L5F1 did not). Similarly, after adjusting arithmetic mean THg concentrations in largemouth bass fillets (**Table A7-9-12**) to whole-body concentrations (whole-body THg concentration = 0.69 x fillet THg; Lange et al., 1998), bass at the majority of the southern sites also exceeded the guidance value for TL 4 fish. However, caution must be exercised in the latter assessment because largemouth bass are considered to be at TL 5 (Loftus et al., 1998). Based on these guidance values, it appears that Everglades populations of piscivorous avian and mammalian wildlife continue to be at risk of adverse effects from mercury exposures. However, as discussed in the next section, levels of mercury observed in great egret tissues do not support this conclusion.

Wading Bird Feathers From ECP Interior Marshes

Results from monitoring mercury exposure of wading birds in downstream interior marshes are summarized in **Table A7-9-16** and **Figure A7-9-18**. To evaluate temporal trends, results from the District wading bird monitoring program are compared to results of studies conducted by Frederick et al. (1997) during the period 1994 – 1995. In accordance with USACOE permit 199404532 Condition 8b.2, these studies were found to be representative of background mercury concentrations in Everglades wading birds (FTN Associates, 1999). These studies involved monitoring THg in feathers of great egret (*Ardea albus*) nestlings at various Everglades colonies. The District’s monitoring program focuses on egret colonies JW1 and L67 located in WCA-3A (**Figure A7-9-5**). These two colonies consistently showed the highest concentrations during the background studies (Frederick et al., 1997, FTN Associates, 1999; Sepulveda et al., 1999).

In 2000, the arithmetic mean feather-THg concentration ranged from 3.2 to 3.4 µg/g dw (**Table A7-9-16**). However, THg concentration in nestling feathers is often dependent on duration of exposure and, thus, age of the bird. Accordingly, concentrations were regressed and standardized for a nestling with a given bill length (i.e., age surrogate) using protocols established by Frederick et al. (1997; note, at that time Frederick standardized mercury concentration to nestling with 7.1 cm bill; **Figure A7-9-18**).

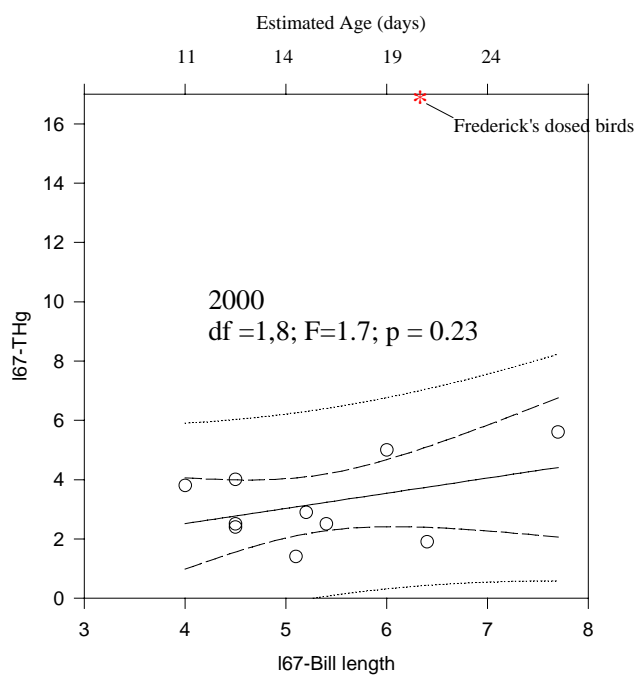
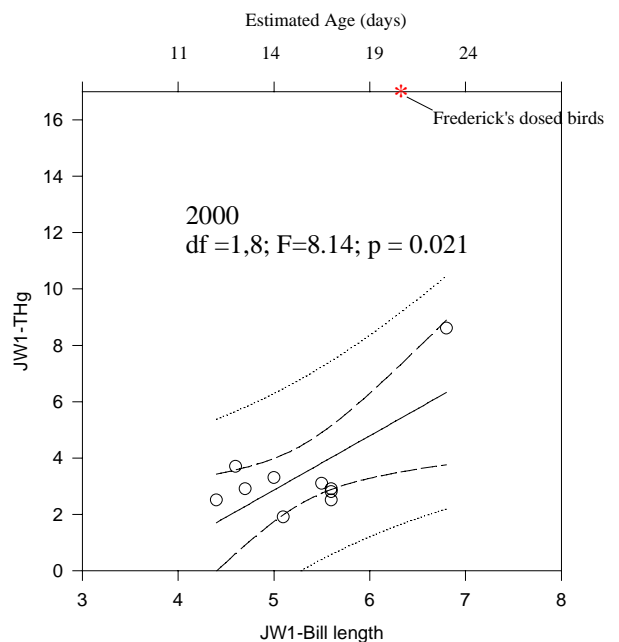


Figure A7-9-18. Relationship between bill length (age surrogate) and THg concentration ($\mu\text{g/g}$) in nestling egret feathers collected in 2000.

Feather-THg concentration in nestlings at JW1 colony (7.18 and 6.9 $\mu\text{g/g dw.}$, **Table A7-9-16**) did not differ between 1999 and 2000 (ANCOVA, $\text{df}=1, 20$; $F=3.04$, $p=0.19$). Because regressions of THg concentration on bill length was not significant for birds from L67 colony in either 1999 or 2000 (ANOVA: $\text{df}=1,18$; $F=2.4$; $p=0.138$; $\text{df}=1,8$; $F=1.7$; $p=0.229$; respectively), standardized concentrations were not calculated nor was ANCOVA used to assess between-year differences. Instead, between-year differences in feather-mercury concentration at L67 (i.e., arithmetic mean concentrations) were evaluated using a Student's t-test and found not to be significant ($t=-0.76$, $\text{df}=28$, $p=0.454$). Furthermore, there was also no between-year difference in bill length in nestlings sampled at L67 (means were 5.5 cm in 1999 and 5.3 in 2000; $t=-0.494$, $\text{df}=24$, $p=0.625$); so even had there been a relationship between concentration and bill length, it is unlikely that LSMs would have differed.

Table A7-9-16. Standardized (least square mean for a chick with a 7.1 cm bill) and arithmetic mean concentrations of THg ($\mu\text{g/g dw.}$) in growing scapular feathers collected annually from of great egret nestlings (2-3 weeks old) at JW1 and L67 colonies.

Colony	LSM \pm 95 th CI (mean \pm 1SD, n)			
	1994 ^{*†}	1995 [*]	1999	2000
JW1	21.12 \pm 6.1 (25.0 \pm 7.9, 9)	14.51 \pm 3.31 (NA, 8)	7.18 \pm 1.14 (4.0 \pm 2.2, 13)	6.9 \pm 1.3 (3.4 \pm 1.9, 10)
L67	16.29 \pm 4.53 (NA, 27)	15.51 \pm 6.16 (15.9 \pm 6.16, 14)	NC (3.6 \pm 1.5, 20)	NC (3.2 \pm 1.4, 10)

* Data from Frederick et al. (1997).

† Concentrations standardized to a bill length of 5.6 cm.

NC – not calculated where slope of regression was not significant ($p > 0.05$).

Estimated mean age of sampled nestling was 16 days in 1994, 24 days in 1995, 15 days in 1999, and 16 days in 2000

Similar to what is reported here, age-related differences in THg concentrations in chick plumage are sometimes conflicting. Several studies report levels to be independent (Thompson et al., 1991, Goutner and Furness, 1997) or even negatively correlated with age (Goutner and Furness, 1997). In the present study, the absence of a relationship between feather-THg concentration and bill length at the L67 colony may be explained by the small sample size ($n=10$) or limited range of ages (based on bill length, chicks ranged in age from about 12 to 23 days). However, when sample size was increased in 1999 by pooling data from an independent concurrent study at this same colony (P. Frederick, pers. communication, total $n=20$), the slope of the regression was still not significant ($p > 0.05$). With regard to range of bill lengths, birds sampled at JW1 colony, which showed a significant regression, had bill lengths similar to birds at L67 (mean bill length at JW1 was 5.6 cm in 1999 and 5.3 in 2000). Monteiro and Furness (1995) maintain that contradictions in age-related differences can be reconciled simply based on level of exposure. They argue that in more heavily contaminated environments, elevated

MeHg exposure to the chick overcomes the natural “growth dilution”, leading to increases in mercury concentration with age. If this is the case here, then it appears that mercury exposure at the L67 site did not keep up with growth dilution.

Although comparisons to earlier surveys is complicated by the lack of standard feather-THg concentrations at L67, it is clear from **Table A7-9-16** and **Figure A7-9-19** that residue levels have decreased in the last two years as compared to 1994 and 1995. This conclusion is consistent with an independent assessment of trends in feather-THg in south Florida egret nestlings by Frederick and Spalding (2000).

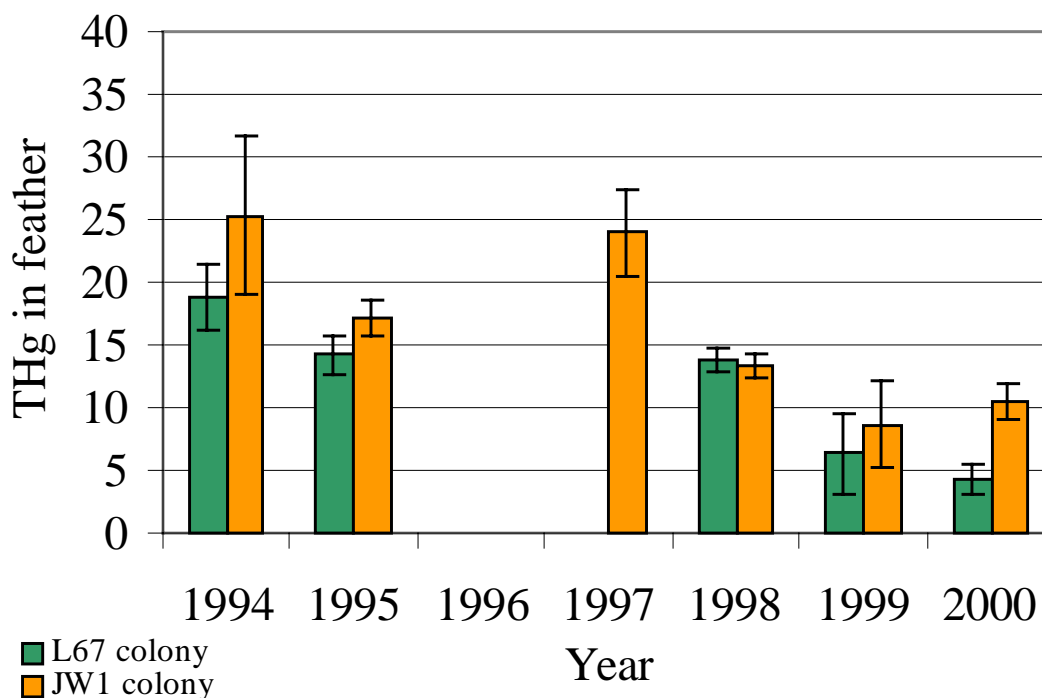


Figure A7-9-19. Total mercury residue trends in egret nestling feathers from select sites (LSM \pm 1SD; $n=8 - 27$; data from P. Frederick, pers. communication; note, Frederick now standardizes LSM to nestling with 8 cm bill length). Data for 1999 and 2000 represent pooled results of feathers collected by the District and Frederick.

Bouton et al. (1999) recently reported results of a controlled dosing study of great egrets that combined feather analysis with toxicological observations. They dosed great egret juveniles (starting at 8 days of age) with MeHg-containing gelatin capsules at 0.5 mg Hg/kg food (n=5) and found subtle behavioral changes and statistically significant differences in blood chemistry, liver biochemistry and weight index (Bouton et al., 1999; Frederick et al., 1979). At three weeks, dosed egret chicks had an average feather-THg concentration of 17 µg/g (Frederick et al., 1997). As evident from **Table A7-9-16** and **Figure A7-9-18**, neither the arithmetic mean nor the least squares mean feather-THg concentration observed in samples collected in 2000 approached 17 µg/g. If we use the value reported by Bouton et al. (1999) as a lowest-observed-adverse-effects (LOAEL) benchmark, then the nestlings at JW1 and L67 colonies do not appear to be at an elevated risk of toxic effects from environmental exposure to MeHg in their diet. However, because Bouton et al. (1999; Frederick et al., 1997) did not begin dosing until day 8, it has been suggested that this LOAEL may be insensitive to possible deficits in early neurodevelopment and, therefore, may not be protective (comments from ECR 2001 Review Panel). Nevertheless, this LOAEL is currently the most appropriate benchmark with which to evaluate results from the District's program to monitoring feather-THg concentration.

In addition to collecting feather samples for compliance with the aforementioned federal and state permits, the District also collected eggs to support an ecological risk assessment of MeHg (for details, see Rumbold, 2000). MeHg is transferred to avian eggs in proportion to dose (Tejning, 1967), and accumulates preferentially in albumen (i.e., egg white proteins; Vermeer et al., 1973, Fossi et al., 1984). Because albumen-mercury is strongly linked to dietary mercury (Walsh, 1990), levels in eggs appear to reflect exposure over a comparatively short period of time, possibly one or two weeks prior to egg laying (Fossi et al., 1984, Furness, 1993). Therefore, depending on the timing of the bird's arrival on the nesting grounds, mercury concentrations in eggs can closely reflect local contamination.

In 2000, egret eggs collected from JW1 and L67 colonies had a mean egg THg concentration of 0.37 ± 0.16 µg/g (fresh weight, n=13). This concentration was not significantly different from concentrations observed in 1999 (0.41 ± 0.205 µg/g fw, n=20; t=0.55, df=31, p=0.59). Alternatively, mean egg-THg concentrations in 1999 and 2000 were lower than concentrations reported for eggs collected throughout WCA-3A in 1993 (mean concentration 0.46 µg/g, n= 43; D. Day, pers. communication); however, without raw data or variance estimates from the 1993 survey, differences cannot be statistically evaluated.

Based on a literature review, Thompson (1996, pg. 345) concluded that "mercury concentrations in eggs of up to approximately 0.5 mg/kg (µg/g) appear to have little detrimental effect on reproduction." Using the mean egg-THg concentration observed in 2000 as a guide, it appears that the risk of adverse effects from current *in ova* mercury exposures to the majority of the egret population is low. This conclusion is consistent also with the risk estimate based on levels of THg observed in nestling feathers.

Interestingly, BMFs for sunfish to egret eggs were small, whereas BMFs for sunfish to egret feathers were large compared to similar indices reported by Hughes et al. (1997) for osprey from the Great Lakes. The BMFs for sunfish (mean concentration from CA3F1, CA3-15, CA2F2) to egret eggs (JW1 and L67) was 1.5 (based on fish collected in late 1998 and eggs collected early in 1999) and 1.6 (based on fish collected in 1999

and eggs collected in 2000). By comparison, Hughes et al. (1997) report BMFs ranging up to 3.71 for yellow perch to osprey eggs. The BMFs for sunfish (from CA3F1, CA3-15, CA2F2) to egret feathers (LSM at JW1) was 26.3 (based on fish collected in 1998 and feathers collected in 1999) and 29.6 (based on fish collected in 1999 and feathers collected in 2000). Hughes et al. (1997) report BMFs for perch to osprey feathers to range from 11.64 to 21.71. A low BMF to eggs (where risk of toxic effects is great) and high BMF to nestling feathers (where MeHg no longer represents a risk) would be an adaptive advantage.

Finally, it is important to place results of monitoring feather- and egg-THg into context with the increases in mercury in fish observed at certain sites in late September – October 1999. Notice that the most recent results reported here were of feathers collected during the 2000 nesting season (March – April). Thus, birds were sampled five to six months after fish. Proper interpretation of the data must consider this time lag. For instance, this time lag might have allowed mercury to propagate further up the food chain into sunfish and bass. This seems unlikely given the observed low concentrations in the birds. Alternatively, if the spike in mercury was short-lived, growth dilution and turnover may have allowed mercury concentrations in the fish to return to steady state. This scenario would be supported by the data. However, these egret colonies are located in central WCA-3A (**Figure A7-9-5**). Therefore, these eggs and feathers may simply confirm what the fish tell us - that central WCA-3A was not as affected by the factor(s) that led to the increased mercury in WCA-2A fish.

Wading Bird Habitat and Foraging Patterns

Various combinations of environmental characteristics determine the suitability of an area for foraging and nesting wading birds. Among others, these characteristics include water depth, vegetation density and, densities and size distribution of the preferred prey populations. These factors have been reviewed in previous reports (Rumbold and Rawlik, 2000). In accordance with Condition (4).iv of the Mercury Monitoring Program, the District conducted a literature search for both published and unpublished studies or monitoring programs that may show possible changes in wading bird habitat and foraging patterns within the Everglades basin during the reporting year. Studies and monitoring programs identified during this search are discussed below.

From February through July 1999, researchers for the USACOE carried out systematic reconnaissance flights (SRFs) for wading bird activity in the WCAs and Big Cypress (Nelson and Theriot, 1999). The ENR and Holey Land were also surveyed. Wading birds were enumerated along parallel transects with 2-km spacing. The SRF survey methodology estimates total numbers of birds on the marsh surface, which is composed of breeding birds out feeding, nonbreeding birds, and juvenile birds. Results from 1999 showed higher numbers of birds in the basin from February-June compared to 1998 (monthly counts ranged from 31,413 to 57,187; all species combined). In 1999, low water levels, with some areas going dry, concentrated birds. For example, estimated numbers of birds in WCA-2A dropped from 12,720 in February 1999 (greater abundance of birds than other WCAs) to just over 1,000 in May. In May, most of the birds within the Everglades basin were found either in WCA-1 (28,627) or WCA-3A (23,533). This dispersal of birds from foraging areas (e.g. WCA-2A, etc.) to nesting areas (e.g., WCA-1 and southern WCA-3A, see below) immediately prior to egg laying increases the spatial scale over which exposure is integrated for the egg (i.e. relative to the nestling).

While total numbers of birds in the WCAs were higher in 1999, spatial patterns were not dissimilar from that observed in 1998 (i.e., relative numbers of birds in the different WCAs). With regard to abundance at the ENR project, mean monthly number of birds was lower in 1999 (72 birds) than previous years (1995: 82 birds, 1996:174 birds, 1997:73 birds, 1998: 23 birds). Thus, although highly variable depending on water levels, wading bird foraging patterns do not show any discernable large-scale shift within the basin.

In 1999, various individuals or agencies also made systematic aerial and ground surveys of nesting wading birds in south Florida (for a more detailed summary, see Gawlik, 1999). In 1999, the estimated number of wading bird nests in south Florida was 27,105 (excluding cattle egrets), which represents a 142% increase over 1998. Numbers of nests in 1999 were similar to 1992, which was the best nesting year in the past 14 years. The vast majority of nests were concentrated in WCAs as opposed to ENP or Florida Bay. In 1999, 11,416 birds were estimated to have nested in WCA-1 (224% increase over 1998). WCAs 2 and 3 combined were estimated to contain 15,273 nests (228% increase over 1998). However, much of the nesting was in WCA-3A, with relatively little nesting in WCA-2A. Only a single colony with less than 100 birds was reported WCA-2A; likewise WCA-2B and WCA-3B contained relatively few nests. Because the Holey Land and Rotenberger went dry, no nesting was reported. Likewise, nests were also not reported for any STA. While systematic ground surveys for nesting were not done in the STAs, any large nesting colony would have likely been observed during routine water quality sampling. Despite increased nesting effort by all species in 1999, only great egrets met the numeric-nesting target set by the South Florida Ecosystem Restoration Task Force.

As of this writing, FFWCC's update to its "Florida Atlas of Breeding Sites for Herons and Their Allies" has not yet been published (S. Nesbitt FFWCC, pers. commun.). FFWCC biologists flew the entire state during the 1999 nesting season recording GPS coordinates, species composition, numbers of nesting pairs and habitat characteristics of nesting colonies. All colonies from previous surveys were checked in addition to newly found colonies. When completed this document will be reviewed for information pertinent to possible changes in wading bird habitat within the basin.

Basic research on the effects of hydrology on wading bird foraging parameters was recently reviewed by Sklar et al. (2000). The lead author of that report is also a co-principal investigator on a study on this subject that is being funded by USGS (Gawlik and Sklar, 2000). Because much of that work was reviewed in the Everglades Consolidated Report 2000 (Sklar et al. Chapter 2 this report), it will not be reviewed here.

In summary, during this reporting year, the District is unaware of any evidence that would support any conclusion that wading bird foraging (or nesting) patterns have been significantly altered or impacted by construction or operation of the STAs or that such changes in foraging patterns would have led to an increased exposure to MeHg via consumption of MeHg-contaminated fish.

KEY FINDINGS AND OVERALL ASSESSMENT

This Report summarizes the first two full years of data from compliance monitoring of mercury storage, release and bioaccumulation in STAs and the downstream receiving waters. Results from this monitoring program describe significant spatial distributions and in some instances between-year differences in mercury concentrations.

Key findings are as follows:

1. During the monitoring period there were no violations of the Florida Class III numerical WQS of 12 ng/L. As such, the project has met the requirements of Section 6.i of the mercury-monitoring program of the referenced permits.
2. This two-year assessment indicates that STA-6 has begun to stabilize with regard to mercury. This was evidenced by: (1) concentrations of THg and MeHg in outflow water less than inflow, and (2) a general decline in THg levels in outflow mosquitofish and largemouth bass. However, because of the continued potential for drydown, STA-6 may never completely resemble the ENR Project with regard to mercury removal efficiency. Discernable differences between treatment cells were identified and will be monitored in the future.
3. While STA-5 and STA-1W both met start-up criteria for operation during the reporting year, neither STA was fully flow-through operational.
4. Atmospheric wet-deposition of THg was lower in 1999 compared to 1998; however, this between-year difference appears rainfall driven (i.e., due to less precipitation in 1999).
5. While highly variable, THg and MeHg concentrations monitored in surface waters at ten Non-ECP structures from May 1997 to April 2000 showed little evidence of significant temporal trends. One location, L28, which drains western watersheds including the C-139 Basin and Big Cypress Seminole Indian Reservation, exhibited an upward trend in THg concentrations that was statistically significant.
6. Mosquitofish collected from interior marsh sites within downstream receiving waters showed significant increases in 1999, ranging from 103 to 1032% increase in tissue mercury concentration compared to 1998.
7. While the magnitude of the between-year difference was smaller and the direction of change was variable among locations, sunfish from at least two interior sites, CA2U3 and L67F1, also showed significant increased mercury concentrations in 1999 over 1998.
8. Between-year differences in standardized age (3) expected mercury concentrations (EHg3) in largemouth bass were generally not statistically significant. However, largemouth bass from one interior marsh site, CA2U3, showed significant increased tissue mercury concentration in 1999 over 1998. Moreover, following FFWCC protocols and standardizing to 3-year-old fish may have masked short-term variations in tissue mercury concentration. Where Year-1 fish were collected, they tended to show high concentrations relative to older cohorts collected in the same year. An

increase in EHg3 bass would be a departure from trends observed by the FFWCC over the last five years that suggest mercury has declined in bass at several sites, including sites within WCA-3A and WCA-2A.

9. Concentrations of mercury in great egret eggs and nestling feathers collected from two colonies within WCA-3A did not differ between 1999 and 2000, and continue to be lower than levels observed during the mid 1990s.

DISCUSSION

Clearly, the observed between-year differences in mercury levels in fish, particularly within WCA-2A marshes, are of considerable ecological interest, if not management concern. Inasmuch as there was no evidence to suggest increased inputs from atmospheric wet-deposition or surface water runoff, proper interpretation of the data must consider other factors that can affect net MeHg production or bioaccumulation.

As previously mentioned, researchers from the USGS reported similar increases in mercury in mosquitofish collected from WCA-2A in October 1999. USGS was in WCA-2A conducting a collaborative study with the District on the effect of sediment drying and fires on mercury speciation and bioaccumulation at 13 sites spanning most of the north-to-south length of the remnant Everglades. WCA-2A has remained flooded continuously in recent years, but following a La Nina-driven dry period, central WCA-2A dried out for 2 months in 1999 beginning in late March (for detailed maps showing areas of drydown see Nelson and Theriot, 1999). While northern WCA-3A also dried out in March 1999, a drydown is typical for this area. Further, about 175,000 acres in the northern WCA-3A burned in late April, 1999. The fires lasted from April 20 to May 12. Although most of the fires were surface fires, which only burned the aboveground vegetation, peat burned in approximately 200 acres of northwest corner. At the time of the first post-burn sampling (July 1999), USGS found levels of MeHg in surface water, porewater, sediment, periphyton, and mosquitofish about 2x, 18x, 11x, 1.5x, and 0.7x higher, respectively, in the burned areas versus non-burned locations. Monitoring at these sites showed that burdens of MeHg in mosquitofish and periphyton continued to build throughout the fall of 1999, reaching maximums in October. Peat oxidation from burning or intense drying could potentially enhance methylation of Hg by increasing the availability of sulfate, labile carbon, Hg(II), or all three. Of these three parameters, USGS found only sulfate at demonstrably higher levels (about 2.4x) in response to the drying and burning.

While the precise biogeochemical mechanism is still uncertain, USGS scientists concluded that drydown, extended dryout and subsequent oxidation (with fires being the extreme oxidation event) altered soil and water chemistry influencing the rate of net methylation of inorganic mercury (discussed in more detail in **Appendix 7-8**). They hypothesize that sulfate was a primary driving factor. Two possible mechanisms may be evoked to explain this increased rate of methylation and it remains to be determined whether only one is important or whether the two mechanisms function simultaneously (their relative importance may differ also spatially). According to one hypothesis, sulfate stimulated the sulfate reducing bacteria (SRB); in the other hypothesis, oxidation of sulfide favored formation of neutral Hg-monosulfide complexes (HgS), which are more available to methylating bacteria (Benoit et al., 1999a). The latter hypothesis is supported by observations that in high-sulfide sediments where charged disulfide complexes (HgHS_2^{-1}) are favored, MeHg production is inhibited (Benoit et al., 1999b,

2000). Following this logic, one could hypothesize that oxidation of organic sulfides to sulfate reduced the charged disulfide complex while increasing the concentration of the uncharged monosulfide, thus releasing any inhibitory pressure. At the same time, methylation would be fueled by increased sulfate. USGS is currently testing these hypotheses by controlled dosing of mesocosms. A corollary to this hypothesis is that the scenario should be reversed upon reestablishment of “normal” redox conditions and the build-up of sulfides produced by the SRB (with disulfide complexes favored over Hg-monosulfide complexes), resulting in reduction in net methylation. As discussed in **Appendix 7-8** (this report), tissues mercury concentrations in mosquitofish began to decline in WCA-2A in November 1999, indicating a rapid clearing of the transient MeHg pulse. What impact this pulse will have on higher trophic levels requires further monitoring.

As was discussed previously, the apparent influence drydown has on sulfide oxidation and mercury biogeochemistry, if substantiated, may have implications for STA-6 that dries out frequently, and the start-up phase of other STAs that are created by flooding oxidized farm peat soils (e.g., STA-1W; see **Appendix 7-14**, this report). Under these circumstances, concentrations of sulfate may be expected to be high but sulfide concentrations in soil pore water are expected to be relatively low immediately following flooding.

The observations over the last year highlights three important points: (1) the mercury compliance monitoring program is sufficiently sensitive to detect interannual differences in food web mercury concentrations, (2) pro-active, adaptive research in the context of a robust research program on mercury sources, biogeochemistry, and bioaccumulation supports a scientifically defensible interpretation of this transient event as being of natural origin and, (3) data from several consecutive years of data will be necessary to clearly distinguish the possible effects of ECP and STA operation from natural influences on mercury methylation and bioaccumulation.

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Table 7-9A-A1. THg concentration (ng/g) and metadata for individual largemouth bass collected at STA 6 in 1999.

Location	Year	Fish No.	Age (yrs)	Length (mm)	Weight (g)	THg (ng/g)	remark
STA6C52	19990907	0250	2	308	568	390	
STA6C52	19990907	0251	1	292	365	97	
STA6C52	19990907	0252	3	315	490	590	
G600	19990907	0253	1	277	313	200	
G600	19990907	0254	2	305	420	420	
G600	19990907	0255	2	336	568	350	
G600	19990907	0256	1	306	354	280	
G600	19990907	0257	1	309	396	210	
G600	19990907	0258	2	312	468	410	
G600	19990907	0259	2	381	734	290	
G600	19990907	0260	1	253	218	240	
G606	19990907	0261	1	341	539	340	
G606	19990907	0262	1	255	221	370	
G600	19990907	0263	2	312	532	250	
G600	19990907	0264	0	278	296	280	
G600	19990907	0265	1	259	225	260	
G600	19990907	0266	0	269	251	260	
G600	19990907	0267	1	305	409	210	
G600	19990907	0268	4	521	2495	570	
G600	19990907	0269	2	322	506	350	A
G600	19990907	0270	2	338	656	280	A
G600	19990907	0271	1	298	388	310	
G600	19990907	0272	1	293	395	270	
G600	19990907	0273	1	244	216	210	
G600	19990907	0274	3	411	1008	510	
G606	19990907	0275	1	254	222	370	
G606	19990907	0276	1	260	234	300	
G606	19990907	0277	2	262	249	590	
G606	19990907	0278	1	361	746	350	
G606	19990907	0279	2	291	313	550	
G606	19990907	0280	1	290	297	400	
G606	19990907	0281	2	291	319	530	
G606	19990907	0282	2	255	203	580	
G606	19990907	0283	4	443	1419	990	
G606	19990907	0284	1	255	239	460	
G606	19990907	0285	2	346	634	500	
G606	19990907	0286	2	312	409	480	
G606	19990907	0287	2	374	754	540	
G606	19990907	0288	2	258	199	930	
G606	19990907	0289	3	405	998	450	
G606	19990907	0290	1	331	539	330	
G606	19990907	0291		248	197	410	age unreadable

Table 7-9A-A2. THg concentration (ng/g) and metadata for individual sunfish collected from ECP and Non-ECP marshes in 1999.

Location	Year	Fish No.	Length (mm)	Weight (g)	THg (ng/g)	remark
WCA2U3	1999	0614	161	73	170	
WCA2U3	1999	0615	138	49	110	
WCA2U3	1999	0616	109	22	97	
WCA2U3	1999	0617	124	32	130	
WCA2U3	1999	0618	111	23	130	
WCA2U3	1999	0619	102	17	120	
WCA2U3	1999	0620	172	97	120	
WCA2U3	1999	0621	170	82	100	
WCA2U3	1999	0622	171	80	83	I
WCA2U3	1999	0623	167	88	100	
WCA2U3	1999	0624	152	63	170	A
WCA2U3	1999	0625	125	33	82	
WCA2U3	1999	0626	148	75	180	
WCA2U3	1999	0627	148	79	220	
WCA2U3	1999	0628	135	53	280	
WCA2U3	1999	0629	125	44	230	
WCA2U3	1999	0630	115	35	270	
L67F1	1999	0631	192	131	200	
L67F1	1999	0632	136	47	520	
L67F1	1999	0633	135	45	510	
L67F1	1999	0634	130	39	610	
L67F1	1999	0635	154	74	340	
L67F1	1999	0636	129	39	390	
L67F1	1999	0637	118	25	410	
L67F1	1999	0638	162	83	1200	
L67F1	1999	0639	139	47	800	
L67F1	1999	0640	162	85	960	
L67F1	1999	0641	137	45	3300	
L67F1	1999	0642	138	52	470	
L67F1	1999	0643	136	43	600	
L67F1	1999	0644	131	41	470	
L67F1	1999	0645	127	55	260	
L67F1	1999	0646	101	24	270	
L67F1	1999	0647	137	46	750	
L67F1	1999	0648	120	31	490	
L67F1	1999	0649	188	110	370	
L67F1	1999	0650	87	14	203	A
CA3F2	1999	0651	157	82	380	
CA3F2	1999	0652	171	121	370	
CA3F2	1999	0653	164	103	360	
CA3F2	1999	0654	155	94	420	
CA3F2	1999	0655	200	184	110	
CA3F2	1999	0656	182	135	130	
CA3F2	1999	0657	157	87	470	A
CA3F2	1999	0658	147	70	89	
CA3F2	1999	0659	102	27	120	
CA3F2	1999	0660	87	14	110	
CA3F2	1999	0661	104	26	210	

Table 7-9A-A2. Continued.

Location	Year	Fish No.	Length (mm)	Weight (g)	THg (ng/g)	remark
CA3F2	1999	0662	92	16	92	
CA3F2	1999	0663	97	22	140	
CA3F2	1999	0664	117	34	150	
CA3F2	1999	0665	101	23	160	
CA3F2	1999	0666	91	18	210	
CA3F2	1999	0667	157	73	310	
CA3F2	1999	0668	137	56	190	
CA3F2	1999	0669	133	48	110	
CA3F2	1999	0670	85	13	120	
CA315	1999	0671	186	148	700	
CA315	1999	0672	198	177	440	
CA315	1999	0673	177	121	650	
CA315	1999	0674	179	114	700	
CA315	1999	0675	174	111	760	
CA315	1999	0676	170	118	360	
CA315	1999	0677	135	64	230	
CA315	1999	0678	122	48	310	
CA315	1999	0679	113	34	230	
CA315	1999	0680	115	33	190	
CA315	1999	0681	104	26	200	
CA315	1999	0682	97	23	350	
CA315	1999	0683	110	23	350	
CA315	1999	0684	111	24	290	
CA315	1999	0685	112	22	250	
CA315	1999	0686	106	22	290	
CA315	1999	0687	107	21	280	A
CA315	1999	0688	105	21	370	
CA315	1999	0689	111	24	220	
CA315	1999	0690	101	20	250	
P33	1999	0691	103	28	420	
P33	1999	0692	102	22	590	
P33	1999	0693	71	7.5	330	
L38F1	1999	0694	192	145	52	I
L38F1	1999	0695	167	96	190	
L38F1	1999	0696	168	93	170	A
L38F1	1999	0697	151	61	150	
L38F1	1999	0698	147	60	110	
L38F1	1999	0699	154	70	61	I
L38F1	1999	0700	153	66	110	
L38F1	1999	0701	198	193	81	I
L38F1	1999	0702	162	90	110	
L38F1	1999	0703	187	144	87	
L38F1	1999	0704	162	89	74	I
L38F1	1999	0705	174	101	84	A
L38F1	1999	0706	196	146	130	
L38F1	1999	0707	185	109	110	
L38F1	1999	0708	137	51	120	
L38F1	1999	0709	172	96	91	

Table 7-9A-A2. Continued.

Location	Year	Fish No.	Length (mm)	Weight (g)	THg (ng/g)	remark
L38F1	1999	0710	149	58	120	
L38F1	1999	0711	141	53	110	
L38F1	1999	0712	132	44	100	
L38F1	1999	0713	147	56	29	I
L5F1	1999	0714	195	131	220	
L5F1	1999	0715	198	161	54	I
L5F1	1999	0716	172	161	53	I
L5F1	1999	0717	172	106	73	I
L5F1	1999	0718	117	24	21	I
L5F1	1999	0719	183	112	59	I
L5F1	1999	0720	213	193	66	I
L5F1	1999	0721	172	100	100	
L5F1	1999	0722	181	112	74	I
L5F1	1999	0723	223	208	62	I
L5F1	1999	0724	153	62	59	I
L5F1	1999	0725	175	94	85	
L5F1	1999	0726	114	33	120	
L5F1	1999	0727	211	183	87	
L5F1	1999	0728	207	163	100	
L5F1	1999	0729	195	121	120	
L5F1	1999	0730	197	121	230	
L5F1	1999	0731	155	71	55	I
L5F1	1999	0732	134	40	27	I
LOX4	1999	0733	191	152	250	A
LOX4	1999	0734	169	141	310	
LOX4	1999	0735	166	121	200	
LOX4	1999	0736	114	28	120	
LOX4	1999	0737	103	19	110	
LOX4	1999	0738	104	19	130	
LOX4	1999	0739	95	14	120	
LOX4	1999	0740	94	14	110	
LOX4	1999	0741	84	9	83	
LOX4	1999	0742	79	11	160	
L39F1	1999	0743	187	135	56	I
L39F1	1999	0744	199	151	110	
L39F1	1999	0745	165	82	46	I
L39F1	1999	0746	169	87	58	I
L39F1	1999	0747	149	65	43	I
L39F1	1999	0748	138	52	100	
L39F1	1999	0749	124	32	70	I
HOLYBC	1999	0750	187	150	41	I
HOLYBC	1999	0751	149	67	46	I
HOLYBC	1999	0752	134	49	67	I
HOLYBC	1999	0753	210	186	41	I
HOLYBC	1999	0754	196	147	41	I
HOLYBC	1999	0755	200	169	55	I
HOLYBC	1999	0756	206	186	47	I
HOLYBC	1999	0757	206	200	59	I

Table 7-9A-A2. Continued.

Location	Year	Fish No.	Length (mm)	Weight (g)	THg (ng/g)	remark
HOLYBC	1999	0758	180	134	32	I
HOLYBC	1999	0759	174	110	24	I
HOLYBC	1999	0760	188	133	36	I
HOLYBC	1999	0761	184	126	29	I
HOLYBC	1999	0762	179	121	33	I
HOLYBC	1999	0763	170	105	28	I
HOLYBC	1999	0764	176	105	67	I
HOLYBC	1999	0765	166	95	23	I
HOLYBC	1999	0766	165	96	33	I
HOLYBC	1999	0767	162	88	41	I
HOLYBC	1999	0768	147	62	24	I
HOLYBC	1999	0769	149	64	34	I
CA3F1	1999	0770	182	95	160	A
CA3F1	1999	0771	171	108	370	
CA3F1	1999	0772	178	119	36	I
CA3F1	1999	0773	181	112	120	
L39F1	1999	0774	146	77	62	I
L39F1	1999	0775	157	79	220	
L39F1	1999	0776	137	55	160	
L39F1	1999	0777	127	40	61	I
L39F1	1999	0778	186	134	45	I
L39F1	1999	0779	173	110	25	I
L39F1	1999	0780	173	103	30	I
L39F1	1999	0781	167	82	38	I
L39F1	1999	0782	179	115	120	
L39F1	1999	0783	182	114	59	I
L39F1	1999	0784	189	161	48	I
CA3F1	1999	0785	197	150	170	
CA3F1	1999	0786	186	132	93	
CA3F1	1999	0787	168	104	66	I
CA3F1	1999	0788	177	108	110	
CA3F1	1999	0789	172	101	120	
CA3F1	1999	0790	142	56	53	I
CA3F1	1999	0791	202	181	140	A
L39F1	1999	0792	200	138	66	I
L39F1	1999	0793	202	163	76	
CA3F1	1999	0798	212	150	83	A
CA3F1	1999	0799	194	132	42	I
CA3F1	1999	0800	168	89	34	I
CA3F1	1999	0801	184	132	130	
CA3F1	1999	0802	167	92	170	
CA3F1	1999	0803	159	74	150	
CA3F1	1999	0804	158	76	81	
CA3F1	1999	0805	162	88	180	
CA3F1	1999	0806	168	91	34	I
LOX4	1999	0807	116	29	97	
LOX4	1999	0808	108	23	140	
LOX4	1999	0809	111	25	110	
LOX4	1999	0810	95	17	77	I

Table 7-9A-A3. THg concentration and metadata for individual largemouth bass collected from ECP and Non-ECP marshes in 1999.

Location	Year	Fish No.	Age (yrs)	Length (mm)	Weight (g)	THg (ng/g)	remark
L38F1	1999	0501	4	420	1153	750	
L38F1	1999	0502	4	327	487	840	
L38F1	1999	0503	4	304	413	600	
L38F1	1999	0504	3	330	549	520	
L38F1	1999	0505	4	364	730	540	
L38F1	1999	0506	3	268	291	450	
L38F1	1999	0507	2	285	289	510	A
L38F1	1999	0508	3	302	421	460	
L38F1	1999	0509	2	242	189	290	
L38F1	1999	0510	2	253	215	380	
L38F1	1999	0511	4	390	713	1200	
L38F1	1999	0512	4	316	478	590	
L38F1	1999	0513	1	255	222	350	
L38F1	1999	0514	1	243	175	250	
L38F1	1999	0515	2	231	154	300	
L38F1	1999	0516	1	225	132	94	
L38F1	1999	0517	0	206	118	200	
L38F1	1999	0518	0	221	142	470	
L38F1	1999	0519	0	208	118	220	
L38F1	1999	0520	1	203	99	120	
CA3F1	1999	0522	4	456	1475	940	A
CA3F1	1999	0523	4	420	1027	1400	
CA3F1	1999	0524	2	337	592	740	
CA3F1	1999	0525	2	323	461	700	
CA3F1	1999	0526	2	307	408	310	
CA3F1	1999	0527	3	382	832	1000	
CA3F1	1999	0528	2	312	432	580	
CA3F1	1999	0529	2	337	504	990	
CA3F1	1999	0530	3	297	361	610	
CA3F1	1999	0531	2	291	389	510	
CA3F1	1999	0532	2	300	364	480	
CA3F1	1999	0533	2	298	370	430	
CA3F1	1999	0534	2	268	269	380	
CA3F1	1999	0535	1	252	216	260	
CA3F1	1999	0536	1	270	260	200	
CA3F1	1999	0537	2	259	223	290	
CA3F1	1999	0538	1	244	211	380	
CA3F1	1999	0539	2	253	175	380	
CA3F1	1999	0540	2	243	160	380	
CA3F1	1999	0541	1	237	160	160	

Table 7-9A-A3. Continued.

Location	Year	Fish No.	Age (yrs)	Length (mm)	Weight (g)	THg (ng/g)	remark
CA3F2	1999	0610	5	415	1122	1100	
CA3F2	1999	0611	1	214	131	370	
HOLYBC	1999	0542	1	270	266	140	A
HOLYBC	1999	0543	5	451	1181	970	
HOLYBC	1999	0544	5	342	592	590	
HOLYBC	1999	0545	4	430	1295	450	
HOLYBC	1999	0546	1	264	246	210	
HOLYBC	1999	0547	5	381	855	450	
HOLYBC	1999	0548	5	310	364	660	
HOLYBC	1999	0549	7	378	821	810	
HOLYBC	1999	0550	5	304	399	400	
HOLYBC	1999	0551	5	325	513	380	
HOLYBC	1999	0552	1	267	244	140	
HOLYBC	1999	0553	6	425	1160	660	
HOLYBC	1999	0554	8	385	771	930	
HOLYBC	1999	0555	2	276	276	140	
HOLYBC	1999	0556	4	390	1018	470	
HOLYBC	1999	0557	4	335	413	490	
HOLYBC	1999	0558	4	311	414	500	
HOLYBC	1999	0559	6	441	1251	610	
HOLYBC	1999	0560	2	302	367	140	
L5F1	1999	0561	5	408	985	310	A
L5F1	1999	0562	3	366	717	830	
L5F1	1999	0563	2	344	526	710	
L5F1	1999	0564	5	314	400	640	
L5F1	1999	0565	5	307	395	690	
L5F1	1999	0566	2	301	363	290	
L5F1	1999	0567	3	271	252	300	
L5F1	1999	0568	2	306	375	170	
L5F1	1999	0569	3	275	274	300	
L5F1	1999	0570	2	279	274	620	
L5F1	1999	0571	3	295	311	490	
L5F1	1999	0572	2	277	271	380	
L5F1	1999	0573	2	281	256	270	
L5F1	1999	0574	2	252	219	300	
L5F1	1999	0575	3	257	195	290	
L5F1	1999	0576	2	242	190	340	
L5F1	1999	0577	2	242	149	320	
L5F1	1999	0578	1	232	147	250	
L5F1	1999	0579		221	131	480	age unreadable
L5F1	1999	0580	1	218	126	300	
L67F1	1999	0581	2	261	229	910	A
L67F1	1999	0582	1	284	295	1200	
L67F1	1999	0583	1	341	518	1600	
L67F1	1999	0584	2	264	232	810	
L67F1	1999	0585	2	234	157	820	
L67F1	1999	0586		218	129	820	age unreadable
L67F1	1999	0587	1	212	117	900	